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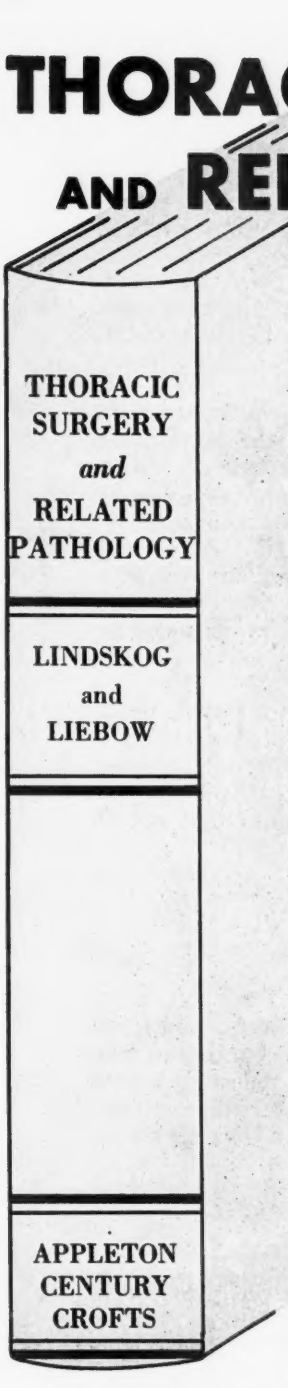

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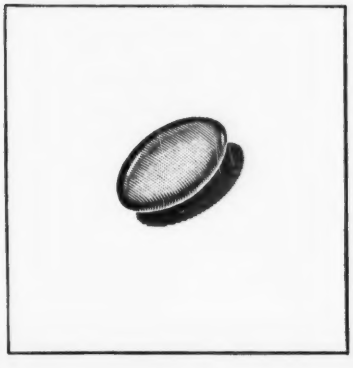

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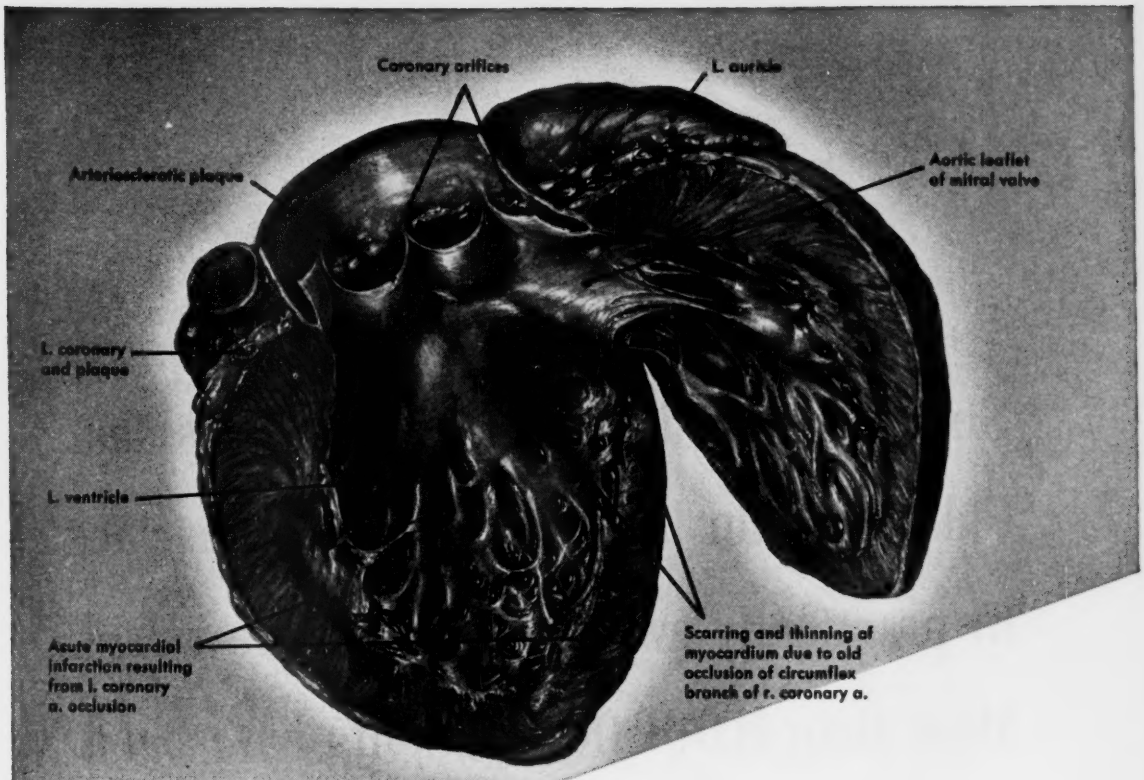
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BACTEREMIA: A CONSIDERATION OF SOME EXPERIMENTAL AND CLINICAL ASPECTS

INTRODUCTION

Invasion of the blood stream in the course of bacterial infection is a dramatic biological phenomenon, the clinical recognition of which has both diagnostic and prognostic significance. Despite the difficulties inherent in separating a single phase of the struggle between parasite and host from the complex process of the pathogenesis of infection, the peculiar clinical importance of bacteremia justifies its discussion as a general process, directing emphasis toward basic mechanisms rather than specific disease entities. It is the purpose of the present communication to review in detail some experimental studies of bacteremia as a basis for an understanding of its clinical significance.

THE ENTRY OF BACTERIA INTO THE BLOOD STREAM

A. *Intravascular Infection*

The simplest form of bacteremia is that which results from the multiplication of bacteria in an area of the body bathed by the circulating blood, an intravascular focus of infection. Obvious examples of this type of lesion are *bacterial endocarditis* and *infected patent ductus arteriosus* or *arteriovenous fistula.* In certain instances, a *mycotic aneurysm* may be an intravascular source of microörganisms. It should be recognized, of course, that these infections are actually not primary processes and that they are the results of previous bacterial invasion of the circulation.

One type of intravascular focus deserves further comment—a *septic* or *suppurative thrombophlebitis.* Infections due to certain types of bacteria or in certain anatomic locations may progress to actual infection of the walls of large veins with thrombosis, colonization of the clot by the invaders, and feeding of bacteria into the blood stream. Organisms likely to produce such infections are the beta-hemolytic streptococcus and members of the group of Gram negative, anaerobic, non-sporeforming bacilli referred to as *Bac-*

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teroides. Bacteroides infections, which are being recognized with greater frequency,[20] are almost invariably characterized by invasion of veins,[60] especially in the regions of the throat and pelvis, the two commonest portals for this organism. Less frequently than in the days before chemotherapy and antibiotics became available, streptococcal endometritis is likely to produce pelvic thrombophlebitis; streptococcal infections of the throat are followed by septic thrombosis of the jugular vein and of the ear by lateral sinus thrombosis.[8, 77] Thrombosis of the angular vein is common in facial erysipelas. Complications of this type are by no means limited to infection by these two organisms; venous involvement may occur with staphylococcal infections also. The anatomic location of a lesion may be a determining factor, as in the case of cavernous sinus thrombosis complicating facial infection or the occurrence of pylephlebitis secondary to intra-abdominal suppuration in the area drained by the portal venous system. A danger in infections of this type is septic embolization. The small infarctions that result from emboli are a fertile breeding ground for bacteria and metastatic abscess formation is common.

Lastly, there are a few circumstances in which, experimentally at least, betahemolytic streptococci apparently enter the blood capillaries directly from the lung alveoli of dogs. Whether this occurs in human lung infections is not known and it was not found to follow instillation of other types of bacteria in dogs.[78]

B. *Extravascular Infection*

Although it is obvious that dissemination of bacteria into the blood stream may be accomplished easily from an intravascular focus of infection, the question of the entry of bacteria from an extravascular site is a more difficult one. The explanation usually offered by textbooks is a general statement to the effect that with severe infection and spread beyond local defenses there may be invasion of capillaries or lymphatics. There is, however, a body of evidence, much of it derived from the studies of investigators whose primary interest was not in the spread of infection, which permits a more precise statement of the probable route of entry of microörganisms into the circulating blood.

Rôle of the lymphatics. Certain characteristics of blood and lymph capillaries deserve emphasis. The typical blood capillary is a tube of endothelial cells connecting the arterial and venous circulations. Its continuous endothelial wall is permeable to crystalloids and smaller protein molecules. The capillary contains blood under pressure which, by appropriate means, can be raised to near arterial levels. The walls of lymphatic capillaries are

composed of very thin endothelial cells. The networks of lymphatic capillaries unite into larger trunks, acquire rudimentary muscle coats, and after interposition of one or more lymph nodes, unite with the venous circulation by way of the thoracic or right lymphatic ducts. In striking contrast to the pressure in blood capillaries, the normal lymph pressure is *zero;* it is well known to investigators who have cannulated the lymph trunks draining an extremity, for instance, that there is no detectable flow of lymph into the collecting vessel unless the extremity is manipulated by brief massage or muscle activity.[15] Lastly, and again differing from the blood capillaries, the endothelial walls of lymphatics are tightly bound to surrounding tissues by reticulum and collagen fibrils,[57] with the result that separation of tissue components by local edema and interstitial fluid accumulation actually holds them open and maintains their patency.

The movement of bacteria in the body has nothing to do with their intrinsic motility. Indeed, most pathogenic organisms are completely lacking in any power to propel themselves and are borne passively in the body fluids. Except for their power to multiply and certain special abilities, such as the elaboration of toxins, etc., they may be regarded as behaving in the body in much the same way as do inert particulate materials such as India ink or colloidal dyes. With caution, one may draw an analogy between certain experimental findings with particulate materials and the movement of bacteria.

There is no question that particulate matter passes through the uninjured walls of blood capillaries. The diapedesis of leukocytes and erythrocytes is familiar enough. Injected particles of India ink pass freely into the tissues from the blood stream.[15] Once a particle has passed out of a blood capillary, it rarely reënters. If one injects a suspension of dye particles into the skin, using a very fine needle and taking care to enter no blood capillary, the dye can be seen to diffuse rapidly into lymphatics.[80] Carbon particles injected intraperitoneally eventually appear in the circulating blood but they reach it by way of the diaphragmatic and mediastinal lymphatics.[9] In this instance, because of the constant rise and fall of the diaphragm, lymph flow is rapid and the particles are detectable in lymph channels within three minutes after injection.[9] Many different particulate materials have been observed to appear in lymph after intravenous injection[83] and such observations might seem to indicate an equal degree of permeability of blood capillaries and lymphatics. In a sense, this is the case, but from the point of view of clearing the tissues of extravascular foreign material, one faces a different set of circumstances. The large size of the lymphatics and the lack of a pressure difference between their contents and the tissues in which they

are found makes them open to the influence of motion or massage and causes them to be the pathways for removal of foreign materials.[15]

Thus far, we have discussed the situation as it obtains with uninjured capillaries and lymphatics. One might reasonably raise the objection that there might be injured or even ruptured blood and lymph channels at the site of a local bacterial lesion. McMaster and Hudack[40] have made observations which bear out the importance of lymphatic channels in removal of particulate materials from a site of injury. These investigators observed under the microscope the course of events after making small incisions or burns in the ear of the mouse. With such injuries, blood capillaries promptly contract, whereas the lymphatic channels remain widely patent for as long as 48 hours. Particulate dyes introduced into wounds were seen to be rapidly taken up through the "gaping ends" of the lymphatics; none entered the closed and sealed capillaries.

There is one other effect of inflammation upon lymphatic function which is noteworthy. With the development of edema, one of the fundamental events in the inflammatory response, there is striking rise in both tissue pressure and lymphatic pressure with consequent increase in lymph flow from the involved area. The classic experiment of Field, Drinker, and White[17] demonstrated that within 2½ hours after immersion of a dog's foot in boiling water, pressure in lymphatics draining the extremity rose from zero to more than twice the venous pressure.

So much for the way in which inanimate particles are cleared from the tissues and for the effects of edema and inflammation upon lymphatic function. What evidence is available from experimental investigations employing bacteria, the specific type of particle with which we are concerned? It will suffice to cite only three of numerous studies[83] which indicate the importance of the lymphatics as a portal of entry of bacteria into the blood stream from extravascular foci.

In 1937, Field and her associates[18] found that rabbits injected intravenously with virulent Type III pneumococci showed these organisms less than one hour later in leg, neck, and thoracic duct lymph. Pneumococci possess no motility of their own; to have reached the lymph, they must have crossed both vascular and lymphatic endothelium.

In 1938, Schulz, Warren, and Drinker[68] cannulated the thoracic duct in rabbits and instilled small amounts of cultures of Type III pneumococci into the trachea, inclining the animal so as to allow the fluid to run down into the alveoli. Organisms were frequently cultured from the lymph within an hour and *while eventually blood cultures often became positive, the lymph cultures were always positive first.*

The interesting researches of Barnes and Trueta[1] must be mentioned. These investigators pointed out that there was doubt in the minds of many workers that bacteria ever enter the blood capillaries directly. It might, however, be imagined that bacteria placed in freshly made wounds could enter the blood stream directly through the open ends of the divided blood vessels, although theoretically this is unlikely because blood is either flowing from them or they are occluded by clot. Using rabbits, Barnes and Trueta exposed the femoral blood vessels and dissected and ligated the lymphatics under a microscope. The muscle, skin and bone were then tied by mass ligation on either side of the femoral artery and vein, leaving the circulation intact as evidenced by active bleeding from freshly made wounds in the calf and by the fact that injection of strychnine into the front of the leg caused death in the afflicted animals as rapidly as in normal controls (strychnine is a drug known to be absorbed directly into the blood stream). Six such experimental animals and 6 controls were then given 0.3 cc. of a saline suspension of *Pseudomonas* bacilli into the skin of the ankle, into the knee-joint or into a deep, fresh wound in the calf muscles. The limb was passively flexed and extended for 30 seconds, and at 10 minutes and 1 hour, blood cultures were taken. With the exception of one experimental animal in which the ligatures slipped, no positive cultures were obtained in the operated group; all controls showed bacteremia. From this and similar experiments, it is clear that even from a recent wound into which an overwhelming number of bacteria has been poured, none are taken up by the freshly injured blood vessels; they appear in the circulation only when lymph drainage from the area is uninterrupted.

Before concluding that bacteria, like inert particles, travel from the tissues to the blood predominantly by the lymphatics, there are at least two possible objections to be met. The first of these is related to the rôle of what Menkin has called "lymphatic blockade" in the localization of bacteria at a site of inflammation.[47] There is no question about the fact that the inflammatory response exerts an influence in preventing the spread of invading pathogenic organisms. All the factors involved in this localization are still not fully understood. Menkin contends that the most important element in local defense is the formation of thrombi in the lymphatics; these thrombi can be demonstrated histologically at inflammatory sites although they are infrequent at the spreading edge of an infection. However, certain other findings and considerations effectively invalidate this as an argument against the spread of bacteria by lymph. First, instead of reduction, there is an increase in the flow of lymph from an inflamed area.[15] Second, Menkin himself has demonstrated that the lymph draining an area of inflammation

contains large numbers of polymorphonuclear leukocytes and, as Rich has pointed out,[61] this finding necessitates that those who uphold lymphatic thrombosis as being significant must argue that there is blockade in the region containing bacteria but none in the region where the leukocytes are located. Miles and Miles[48] believe that much of the "fixation" of various substances, such as dyes, at inflammatory sites is due to dilution by the accumulated exudate such that, despite increased lymphatic drainage, there appears to be retention of the foreign material. Lastly, the simple clinical observation of the frequent swelling and tenderness of regional lymph nodes with local infection argues against any complete lymphatic blockade.

A second possible objection is that lymph nodes filter bacteria and other noxious materials from the lymph stream. There has never been demonstrated a pathway for lymph from the peripheral tissues to the blood stream which avoids passage through at least one lymph node.[15] These nodes are extremely effective, although by no means perfect bacterial filters. Drinker *et al.*[14] observed the filtration efficiency of lymph nodes to be 99 per cent for streptococci but concluded that the protective action of a node may break down when the number of bacteria reaching a node is excessive, when the perfusion pressure of the lymph becomes too high, when the organism is unusually virulent, or when the node is subjected to massage. The protective rôle of the lymph nodes, therefore, does not offer an insuperable objection to a lymphatic route of spread, particularly if one considers recent studies by Smith and Wood[70, 71] who have shown that the filtering action of *normal* lymph nodes is inefficient in that the nodal sinuses possess relatively few cells capable of phagocytosis. However, during active infection, there is also infection of the node and, within a few hours, there occurs an explosive inflammatory response with outpouring of polymorphonuclear leukocytes into the sinuses. The node is thus transformed into a highly efficient filter. In the early stages of infection, there is good opportunity for the infected lymph stream to reach the blood.

It seems clear then, that the lymphatics play a primary rôle in the entry of bacteria from extravascular foci of infection into the blood stream and the production of bacteremia. Later, in discussing clinical aspects of bacteremia, there will be occasion to refer to some of the characteristics of lymphatic function.

Transport by polymorphonuclear leukocytes. The facts that many bacteria are engulfed by polymorphonuclear leukocytes at a local inflammatory site and that the filtering action of lymph nodes is in such large measure dependent upon phagocytosis by polymorphonuclear leukocytes led to consideration of another mechanism for entry of bacteria into the blood stream,

namely the reëntry directly into blood capillaries of leukocytes which have ingested organisms. Drinker has demonstrated the occurrence of this in the capillary circulation of lymph nodes.[15] Although most bacteria taken up by phagocytes are destroyed by intracellular digestion, as Topley and Wilson[83] put it, "the tacit assumption that a bacterium phagocytosed is, of necessity, a bacterium finally disposed of is certainly unwarranted." There is excellent evidence that phagocytosis protects some bacteria,[22, 65] and recent studies by Rogers and Tompsett[69] indicate that pathogenic staphylococci can survive ingestion by human leukocytes. Wilson[82] has shown that streptococci are often ingested and then egested by leukocytes, retaining ability to multiply.

FATE OF BACTERIA IN THE BLOOD STREAM

A. *Factors Influencing the Removal of Injected Bacteria*

The simplest method for experimental study of the fate of microörganisms which enter the circulation is the injection of bacteria directly into the blood stream of an experimental animal. Shortly after a single intravenous injection of viable bacteria, the blood culture of an animal contains many colonies of the organism. When serial cultures are taken, the colony count is found to decrease precipitously and, with many bacterial species, the blood stream becomes sterile within a period of one to several hours. Numerous investigators, beginning with Wyssokowitsch in 1886,[88] have demonstrated this rapid removal of injected bacteria from the blood.[58] The factors which influence disappearance rate and completeness of removal have been studied extensively. Although there have been minor variations in the results obtained with different animals and bacteria, the operation of an efficient mechanism for clearing bacteria from the circulation is easily and consistently demonstrable.

The *virulence* of the injected bacteria is important. The extensive studies of pneumococcal infection by Wright[87] included observations of the number of pneumococci in blood cultures of rabbits at intervals after intravenous injection of an avirulent, a moderately virulent, or a highly virulent strain of this organism. The avirulent strain was rapidly and completely removed from the blood, all cultures being sterile after five hours. Injection of moderately virulent pneumococci was followed by a tremendous fall (99%) in colony count during the first five hours. This initial decrease in the number of organisms which could be cultured from the circulating blood was followed by a transient rise at 24 hours, presumably due to the ability of this strain to multiply in the host's tissues and to reinvade the blood. The circu-

lation, however, finally became bacteria-free and remained so. Virulent pneumococci were also cleared rapidly from the blood stream during the first five hours after injection, but the bacterial count promptly returned to high levels as this strain began to multiply and to invade, and all animals died with sustained bacteremia. These examples from the experiments of Wright are representative of the pattern of events observed by many other investigators.[83] The number of microörganisms in the circulation at a given time is a balance between multiplication, invasion, and the clearing mechanism. Evidence that the ability to remove bacteria is retained even in fatal infections is the fact that injection of an excess of organisms into the blood stream of an animal dying with an infection due to the same bacteria is followed by removal of the injected microbes at a normal rate. This has been demonstrated by several investigators with different bacteria.[6, 27, 59]

Before specific mechanisms of bacterial clearing are described, certain factors which modify the disappearance rate after a single injection of a suspension of organisms may be mentioned. The disappearance of injected bacteria, pathogenic or non-pathogenic, is accelerated in the presence of *specific antibody,* whether present by active or by passive immunization.[11, 73, 74, 87] The injection of virulent bacteria into the immune animal results in a disappearance curve resembling that for avirulent bacteria in the normal animal. Furthermore, Wright found that prior contact with the organism resulted in enhanced clearing of the blood stream in animals before specific antibody became demonstrable.[87]

The production of *severe leukopenia* (by benzol injection) does not influence the rabbit's ability to remove injected pneumococci from the blood.[87] Injection of India ink or lithium carmine particles in attempts to *blockade the "reticulo-endothelial" cells* did not influence removal rate of pneumococci in Wright's experiments. These experiments have never been repeated using Thorotrast, a potent blocking agent for the fixed macrophages.

One of the striking effects of cortisone and ACTH upon experimental bacterial infections has been the tremendous increase in mortality in animals given these hormones and the presence of high levels of bacteremia in these animals.[29] Germuth, Ottinger, and Oyama[24] have demonstrated, however, that the increased bacteremia in cortisone-treated rabbits infected with pneumococci is probably not the result of any interference with the clearing mechanism but rather represents some enhancement of multiplication of organisms or their entry into the circulation. The reasons for the increased mortality from infection in animals pretreated with adrenal steroids are far from clear.[29]

Recent studies by Stavitsky[72] have shown that the production of an anaphylactic reaction impairs the ability of rabbits to clear the blood of injected dysentery bacilli.

Where do bacteria go when they leave the circulation? Briefly, on the basis of numerous cultural and histological studies, it can be said that the majority of bacteria cleared from the blood after a single intravenous injection can be found in the liver, the spleen, and, in certain instances, the lung.[11, 58, 73, 74]

B. *Rôle of the "Reticulo-endothelial System" (RES) in Removal of Bacteria from the Blood*

It is well known that particles of India ink, various dyes, and colloidal metals are not excreted after injection but are deposited in various organs where they are stored in macrophages which have a characteristic distribution throughout the body. These fixed phagocytes, the Kupffer cells of the liver, the lining cells of the splenic sinusoids and sinuses of lymph-nodes, and the histiocytes of the bone-marrow, possess the common characteristic of storing dyes and particulate matter and probably constitute an integrated, functional system. It is in the macrophages of the RES in the liver and spleen that most bacteria removed from the blood after a single injection are found.[11, 41, 58, 73, 74] Organisms occasionally cultured from the lung are apparently trapped and ingested by polymorphonuclear cells in the alveolar capillaries.[73, 87] The rôle of the lung in clearing the blood stream as well as the part played by polymorphonuclear leukocytes will be discussed in a later section.

The importance of certain tissues in maintaining the balance between multiplication of bacteria in the tissues, invasion of the blood stream, and the operation of a clearing mechanism in human bacteremia was demonstrated by the investigations of Beeson, Warren, and Brannon[4] in patients with bacterial endocarditis. The experiments involved multiple simultaneous cultures of arterial and venous blood. Arterial samples were collected from the femoral artery and venous samples were collected from several sites by means of an intravenous catheter, including right atrium, renal vein, and hepatic vein. It was noted first that the bacterial count in arterial blood in this disease remained remarkably stable over periods of a few hours. (This is in accord with findings of previous workers who obtained cultures every few hours for several days.[80]) A significant reduction in the number of organisms in pooled central venous blood from the right atrium as compared with arterial counts was consistently demonstrable. No evidence was found of removal of bacteria from blood circulating through the extremities

or kidney, but as blood passed through the liver, a dramatic reduction in bacterial count occurred. Hepatic venous blood always contained many fewer colonies than arterial or other venous samples. Two conclusions can be drawn from these experiments. First, in bacterial endocarditis, the bacteremia was shown to be maintained at a relatively constant level, eliminating the idea that bacterial invasion of the blood stream in this disease consists only of irregular spurts and showers of organisms as tiny bits of valvular vegetations break off—there is a *steady* feeding of organisms into the blood. Second, the operation of a clearing mechanism through the liver in human bacteremia was demonstrated, furnishing a firm basis for drawing conclusions from animal experiments pointing to a rôle of the RES.

Using a modification in animals of the catheterization technique, Martin, Kerby, and Holland have conducted an extensive series of studies of the removal mechanism in rabbits and dogs. Because the hepatic veins drain not only the liver, but also the spleen and a portion of the gut, these workers prefer the term "splanchnic" removal of organisms. Essentially, their technique has been to obtain simultaneous arterial and hepatic venous cultures for comparison of colony counts.[45] Bacteremia is maintained by a constant intravenous infusion of organisms, thus more nearly duplicating the situation in most clinical infections than does a single injection of a bacterial suspension. The findings of these investigators may be summarized as follows:

(a) For a given organism there is a remarkably constant per cent removal rate.[33] This allows accurate comparison of results in normal animals with those in animals subjected to various types of treatment or manipulation.

(b) Removal rate varies with the species of organism studied. Staphylococci are rapidly removed, encapsulated Friedlander's bacilli less rapidly, and virulent pneumococci slowly.[33] Non-mucoid Friedlander's bacilli are removed more rapidly than the encapsulated variant of the same species.[32]

(c) Specific antibody enhances splanchnic removal of bacteria.[33]

(d) Whole body x-ray irradiation or radiation localized to the liver and spleen exerts no effect upon the clearance rate in the splanchnic area.[10]

(e) Animals made granulocytopenic by nitrogen mustard show normal splanchnic clearance of infused bacteria.[34]

(f) The prior injection of Thorotrast, a colloid which localizes in the cells of the RES (so-called "blockade"), depresses the rate of splanchnic clearance.[46]

(g) The splanchnic clearance rate is uninfluenced by large doses of ACTH or cortisone.[44] This finding, with those of Germuth, Ottinger, and

Oyama[24] mentioned earlier, indicates that the enhancement of infection by adrenal steroids is due to some effect upon the infection in the extravascular tissues.

(h) In the course of fatal pneumococcal bacteremia in rabbits, the increased number of bacteria which appear in the circulating blood is not due to any overwhelming of the splanchnic clearing mechanism but is a result of increased multiplication and invasion by the organisms. If staphylococci are infused intravenously into an animal dying with pneumococcal bacteremia, the staphylococci are removed in the splanchnic area at a normal rate.[43]

These studies confirm the ability of the RES to remove bacteria from the blood and demonstrate that its function is extremely stable. However, all of the observations were limited to the splanchnic clearing mechanism and the effect of such procedures as injection of Thorotrast, etc. upon the total ability of the blood to remove bacteria after a single intravenous injection is not known. It is conceivable that other mechanisms of bacterial removal might enable clearance to continue at a normal pace despite depression of the splanchnic removal by a "blocking" agent.[86, 97] Indeed, the studies of Narita[51] suggested that this may be the case although he used India ink rather than the more effective Thorotrast to produce blockade of the fixed macrophages.

The removal of bacteria by the RES is easily demonstrated and has been studied extensively. That it is of importance in infection can hardly be questioned. Other factors, however, may be of equal importance in bacterial removal although our knowledge of these other mechanisms is still incomplete.

C. *Rôle of the Polymorphonuclear Leukocyte in Clearing the Blood Stream*

Although most of the bacteria removed from the circulation after intravenous injection are found in the liver or spleen, the lung is regularly found to contain an appreciable number of organisms. Histologically,[85, 97] many of these are found to be ingested by polymorphonuclear leukocytes within the alveolar capillaries. Several workers have described phagocytosis of bacteria by granulocytes in the capillaries of other organs also.[75] Subsequently, many of these cells are probably transported to the phagocytes of the RES where final disposition takes place. Wood, on the basis of further studies of intravascular phagocytosis, has suggested that the ingestion of bacteria in the circulating blood serves as an important defense of the host in acute bacteremia.[96] Observations were made on the intravascular reaction to injection of pneumococci or Friedlander's bacilli by employing the rabbit ear-

chamber. Following injection of the bacterial suspension, the polymorphonuclear leukocytes were seen to stick to the endothelium of capillaries and to become motile, migrating freely about the endothelial surfaces. Within 15 minutes, ingestion of bacteria by these cells began to occur by the mechanism which Wood has called "surface phagocytosis," a process which is possible in the absense of specific antibody or "opsonin."[85] The relative importance of the RES and the polymorphonuclear leukocyte in removing bacteria from the blood cannot be evaluated on the basis of the evidence now available. Although destruction of leukocytes by benzol[87] or nitrogen mustard[84] does not alter clearance rates, the effect of RE blockade upon total clearance is not known. Wood has suggested that the peripheral leukocytes and the macrophages of the RES supplement one another in clearing the blood of bacteria and that impaired function of one system is quickly compensated by the other mechanism.[86] Further experimental observations will be needed to clarify this relationship. The observations of Gregg and Robertson[25] on dogs with bacteremia due to the pneumococcus suggest strongly that intravascular phagocytosis by polymorphonuclear leukocytes is of great importance in clearing pneumococci from the blood stream in this animal.

The finding of Stavitsky,[72] mentioned earlier, that mild anaphylaxis results in prolongation of the time required for clearing of injected dysentery bacilli from the blood of rabbits is of interest in connection with the possible rôle of polymorphonuclear cells in bacterial removal. Among other changes which accompany anaphylactic shock, severe neutropenia is regularly present.

Despite the gaps in our knowledge concerning the exact mechanisms and the relative importance of clearing of bacteria from the blood by the cells of the RES and peripheral polymorphonuclear leukocytes, it is clear that in most instances the blood stream is endowed with powerful antibacterial defenses. The poor prognostic significance of bacteremia in the course of infections is not due to multiplication of bacteria in the blood stream, but is important because it reflects the failure of localization of infection at the extravascular site with subsequent spread and invasion of the blood. Illustrative of the great ability of the blood to resist infection as compared to other tissues of the body are many experiments such as those of Lange and Gutdeutsch who showed that it is far more difficult to establish fatal infections in mice by injecting pneumococci or streptococci intravenously than when the intraperitoneal or subcutaneous route is used.[83]

In summary then, we may conclude that there is a normal, efficient clearing mechanism for bacteria which enter the blood stream, and that this mechanism is in part a function of the fixed macrophages of the RES

and also of the circulating leukocytes. Specific antibody enhances bacterial removal and other agents may depress it. The level of bacteremia in any given infection represents a balance between bacterial multiplication, invasion of the blood stream, and the activity of the clearing mechanism.

SOME CLINICAL CONSIDERATIONS

A. *Types of Bacteremia*

Clinically, bacteremias are of two types, *continuous* and *intermittent.* Continuous bacteremia is always a sign of serious infection.[31, 54] Bacterial endocarditis has been mentioned already. Bacteria are constantly present in the blood during the first week of typhoid fever, and brucellosis may be accompanied by bacteremia which persists for several weeks.

In contrast to these severe, continuous bacteremias are those of the intermittent variety. Transient bacteremia probably occurs in nearly all patients with pneumococcal pneumonia at the beginning of the disease, hence the typical onset with shaking chill. In most cases, this spill-over of organisms from the lung is promptly dealt with by the clearing mechanism as the infection in the lung localizes. However, 20 to 30 per cent of patients with pneumonia are bacteremic when hospitalized.[13] As is well known, the later in the course of pneumonia that organisms can be grown from the patient's blood, the more guarded is prognosis.[13] The presence of organisms in the blood is simply an excellent indication that there is spreading infection in the lung.

There are many types of transient bacteremia which are of little consequence. Most of these are the result of a sudden shower of organisms into the blood stream. Clearing is usually rapid and the episode is often unaccompanied by characteristic chill and fever. In the majority of instances, these transient bacteremias follow manipulation of infected or contaminated tissues.

Instrumentation of the genito-urinary tract, even so simple as the insertion of a urethral sound, may be followed by chill and transient fever.[2, 53] Failure to obtain positive blood cultures at the time of the chill led for many years to the belief that some peculiar property of the urinary tract was responsible for so-called "catheter fever." However, when the lag-period between bacteremia and chill is allowed for, and blood is drawn immediately after instrumentation, in a large number of cases organisms can be cultured.[2]

Schottmüller[67] observed large numbers of bacteria, as many as 1,000 per cc., in the circulation immediately after *uterine curettage* for septic abortion. The blood was invariably sterile 15 minutes later. Büngeler[7] described

hematogenous dissemination of tuberculosis after curettage for tuberculous endometritis. Bacteremia has been observed to accompany the pelvic turmoil of normal labor; and Kulka, who took blood cultures at regular intervals in a number of normal women, found frequent, asymptomatic bacteremias during their menstrual periods.[36]

Several types of operative procedure may be accompanied by bacteremia. As examples from numerous studies, Elliott[16] found 38 positive cultures among 100 patients bled within five minutes after *tonsillectomy*. All were negative at 15 minutes. Similarly, Fischer and Gottdenker[19] found 21 positive blood cultures among 64 patients immediately after tonsillectomy. Likewise, *dental manipulations* often lead to spill-over of organisms into the circulation.[5, 52, 64] Positive blood cultures have been obtained after extraction of teeth and even after such a procedure as simply chewing paraffin for 30 minutes.[50] Careful studies indicate that the incidence of bacteremia following intraoral procedures is more closely related to the degree of trauma than to actual intensity of oral sepsis.[38] Seifert[60] cultured bacteria from the blood 10 minutes *after incision of an abscess* in 45% of his patients. Richards[62] obtained positive blood cultures in 15% of 376 patients after simple massage of infected foci such as furuncles. Lastly, the study of Reith and Squires[58] should be mentioned. These workers took blood for culture at random intervals over a period of months from 293 subjects of whom 278 were males. They obtained one or more positive cultures in 65 of these normal individuals and observed that positive cultures were far more frequent during months of the year when upper respiratory infections were common.

The relationship of many of these transient bacteremias to massage or manipulation is strongly reminiscent of the procedures used experimentally to stimulate the flow of lymph. Their significance, of course, lies in the important relationship they are now known to bear to the pathogenesis of bacterial endocarditis in patients with residual valvular damage. Tonsillectomy, dental procedures, normal labor, and genito-urinary instrumentation are now widely recognized predisposing causes for endocarditis. Fortunately, premedication with penicillin will largely (although not entirely) prevent these bacteremias[26] and in patients with known valvular heart disease it should certainly be used. Because of the type of organism likely to be encountered in the genito-urinary tract, aureomycin or some similar drug is probably preferable.

B. *Blood Cultures*

Can we draw any conclusions about taking blood cultures for diagnosis? In the patient with intermittent chills blood for culture should be obtained

during the hour preceding the expected chill or spike in temperature.[23, 35, 80] This is important because there is a lag period of about one hour between the time of the sudden influx of bacteria and the onset of the rigor, and the blood is often sterile by the time fever begins. In patients with suspected endocarditis, rather than many cultures in one day, a few cultures daily for several days is the proper schedule. It is striking to note that blood cultures in this disease are usually *all* positive or *all* negative—rarely does one see an isolated positive culture in this disease. It is not meant to imply that the taking of many cultures at frequent intervals is not indicated in febrile patients in whom the diagnosis is obscure. In many cases of chronic meningococcemia, for example, the organism can be obtained only after many cultures have been made.

The question occasionally arises of obtaining *arterial blood* for culture when ordinary venous blood cultures are negative. Although it has been stated, without adequate demonstration, that this procedure may offer diagnostic advantages,[49] the balance of experimental and clinical evidence indicates that there is no removal of bacteria in the circulation of the extremities. Consequently, blood from the antecubital vein is as likely to contain organisms as blood from the brachial or femoral arteries.[4, 80]

In certain infections, culture of the bone marrow may give superior results.[5] This is particularly true in typhoid after the first week of the disease and in histoplasmosis; occasionally it is helpful in brucellosis. The superiority of marrow culture in these diseases probably lies in the fact that these organisms survive for long periods of time in macrophages, and in the marrow one taps a richer source of these cells than in the peripheral blood.

In rare instances, direct observation of a stained smear of peripheral blood or the buffy coat[28] may reveal organisms. Meningococcemia, in which enormous numbers of bacteria may be present, is the commonest disease in which this procedure is helpful. It is estimated that when organisms can be seen on direct smear, the colony count is at least 100,000 per cc. The tremendous number of meningococci which may be present in the peripheral blood raises the possibility that in this infection, actual multiplication of organisms in the blood stream may occur, although this has not been established. Of course, in histoplasmosis or relapsing fever, the causative organism may be first found in the peripheral blood smear.

What is the significance of colony counts in clinical blood cultures? It is obvious that invasion of the blood stream by bacteria is per se a benign event as compared to invasion of other tissues such as the peritoneum. Unless the colony count rises to several thousand per cc., few conclusions

can be drawn other than the general conclusions already discussed—10 or 200 pneumococci per cc. of blood have the same meaning. It is of interest that the actual number of typhoid bacilli present in the blood, for instance, is rarely more than 30 per cc. and is usually less than 10.

C. *"Treatment" of Bacteremia*

Contrary to statements advocating the immediate administration of penicillin or some other antibiotic *intravenously* if bacteremia is present, therapy should be directed at the primary focus of disease.[31] As we have seen, of all tissues which display defensive powers, the blood stands at the top. Suppose we are told that there are 500 colonies of pneumococci per cc. of blood? Consider the millions of organisms present in a single cubic centimeter of the infected lung and direct therapy to the real source of trouble.

D. *Bacteremia and Hemolysis*

It is often stated that hemolytic streptococcal infection accompanied by bacteremia results in severe anemia due to hemolysis. This is not the case as should be obvious from the lack of signs of rapid blood destruction such as jaundice or hemoglobinuria. Although some severe staphylococcal or salmonella infections are accompanied by icterus, the bilirubinemia is a result of toxic hepatitis rather than hemolytic action. Bacteremias due to *Clostridium welchii,* however, are characterized by rapidly developing icterus, hemoglobinuria, and severe anemia; the development of such a picture should raise the possibility of this infection.

E. *Bacteremia and Lumbar Puncture*

A question which commonly arises relates to the possibility of producing meningitis by performing lumbar puncture upon a patient with bacteremia. Although under very special experimental conditions in animals this is possible,[78, 79] there is no evidence that it is of clinical importance[56] and it should not deter us from spinal fluid examination when this is indicated.

F. *Bacteremia and Specific Antibodies*

With the exception of brucellosis, the demonstration of specific antibody against an organism at a time when that bacterium is cultured from the blood is pathognomonic of endocarditis (or some more unusual intravascular forms). As an example, the demonstration of the Quellung reaction with a patient's serum against pneumococci cultured from his blood means

endocarditis. However, the antibodies must be shown to be present when the culture is *drawn,* not when it is reported positive a few days later.

G. *Negative Blood Cultures*

Negative blood cultures are often as much of a problem to the clinician as positive findings. In many patients with bacterial endocarditis, there is little doubt that the number of bacteria entering the blood stream may be so small and so easily dealt with by the clearing mechanisms that positive cultures are not obtained.[30] Such patients may die, ample evidence of the relative unimportance of bacteremia itself as compared to the original focus of infection in the heart valve. In 1908, Dr. Emanuel Libman[57] pointed out that there are a number of bacterial infections in which it is unusual to find positive blood cultures. Among those listed were liver abscess, cholecystitis, appendicitis, peritonitis, and pylephlebitis. The striking thing in common among these is the fact that all are located within an area drained by the portal venous system. It seems possible that any overflow is promptly cleared by the RES of the liver before reaching the systemic circulation. In the case of pylephlebitis, organisms enter the portal blood directly. However, the probability is that any entry of organisms into the blood from the others is via the lymphatics and other factors are responsible for the infrequency of positive blood cultures.

H. *Bacteremia in Cirrhosis*

In some patients with Laennec's cirrhosis, there occur spontaneous bouts of bacteremia due to organisms which normally inhabit the intestine, usually the colon bacillus.[81] One possibility offered to explain this occurrence is that there is normally some passage of intestinal organisms into the portal circulation and that the normal liver promptly removes these bacteria from the blood. In the cirrhotic, however, if this be the case, there are two possible explanations for failure of a normal clearing mechanism. The first is that there is some impairment of function of the RES as well as the parenchymal cells in cirrhosis. A second alternative is that with the occurrence of portal hypertension and the development of shunts (such as esophageal varices) around the liver, blood containing bacteria simply fails to pass through this organ and bacteremia occurs. The comprehensive study of Sborov *et al.*[98] may help with the question of possible escape of intestinal bacteria to the liver in man. Although it is well known that this occurs in dogs,[12, 42, 84] Sborov, who cultured portal venous blood and biopsy specimens from more than 60 patients, found only one positive culture; interestingly, this patient

had cirrhosis of the liver. Therefore, while it seems unlikely that the normal liver is called on to filter out intestinal flora in human subjects, it is within the realm of possibility that there is some leakage of organisms into the portal vein from the gut in patients with cirrhosis and that these bacteria escape the normal clearing mechanism in some way. The failure of Sborov to find bacteria in portal venous blood is in accord with the probability that the entry of most organisms into the blood stream is indirect by way of the lymphatics rather than directly into the circulation.

I. *Bacteremia and Diphtheroids*

Lastly, there may be mentioned the possible significance of finding organisms usually regarded as contaminants in blood cultures, the so-called diphtheroids. It has been reported by Fleisher[21] that a very high percentage of blood cultures containing diphtheroids are found to come from patients with malignant lymphoma and other chronic febrile disorders. Fleisher does not postulate a causal relationship, rather he simply suggests that diphtheroids in a blood culture should bring to mind these diagnostic possibilities. We have studied a case in which diphtheroids were cultured repeatedly from blood and bone marrow of a patient found at autopsy to have periarteritis nodosa. Further observations are needed regarding the significance of this kind of bacteremia.

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THE ACUTE EFFECTS OF PURIFIED CRYSTALLINE PITUITARY GROWTH HORMONE IN NORMAL HUMAN BEINGS*

The ability of purified crystalline preparations obtained from the anterior pituitary to produce growth in hypophysectomized animals has been established repeatedly.[30, 32] The effectiveness of a given preparation can be demonstrated by its ability to produce linear growth[48] or to produce a positive balance of nitrogen.[40] The production of an increased epiphyseal width is also considered evidence of growth-promoting activity,[23] although this effect has also been reported as a result of the administration of insulin[41] or glucagon.[12] In human beings, the production of growth or of epiphyseal changes cannot easily be studied, and most investigations of the effects of the hormone have therefore been confined to balance experiments. Attempts to produce a positive nitrogen balance in normal, diabetic, or hypopituitary human patients have been unsuccessful[2, 10, 13 29, 33] or incompletely reported.[8, 37] The difficulties of conducting accurate balance studies in human beings are so great that only relatively large changes can be measured. Consequently, physiologically significant alterations may have been produced in human experiments, but the effect may not have been recognized because of the grossness of the technique. The available preparations of growth hormone have also usually produced untoward effects after several days of administration to human subjects, so that the anabolic actions may have been obscured by the side effects such as fever, nausea, and anorexia.

In addition to its well-established effects in promoting growth, the hormone also produces certain acute alterations in the metabolism which can be detected in relatively short-term experiments in animals. For example, the hormone causes an acute fall of blood glucose in intact rats[35] and dogs,[26] a fall of plasma α-amino nitrogen in rats[16, 17, 35] and in the blood amino nitrogen of cats,[34] and a rise of ketone bodies of rats.[1, 3, 21] In hypophysectomized rats, a rise of plasma inorganic phosphate occurs after the administration of growth hormone.[31] Anti-insulin and diabetogenic properties may

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also manifest themselves after short periods of treatment in animals.[9, 27] Since these alterations are probably linked in some way with the growth-promoting effect of the hormone,[45] and since it seemed likely that the acute changes would be easier to demonstrate in human subjects than long-term changes of nitrogen balance, attempts were made to reproduce in human beings some of the metabolic effects of the acute administration of growth hormone in experimental animals.

METHODS

The subjects consisted of 13 volunteers who were either entirely normal or were convalescing from a minor acute illness. The experiments were conducted after an overnight fast, with the subjects lying at ease on a bed in a quiet room. All venipunctures were made under epinephrine-free procaine local anesthesia. In most cases samples were withdrawn from a Cournand needle set in place in an antecubital vein before the beginning of the experiment so that repeated venipunctures were avoided. No sedatives were used.

The growth hormone* was prepared according to the method of Wilhelmi *et al.*[47] and was supplied in lyophilized form in sterile bottles containing 25 mg. each. The contents of each bottle were dissolved in five ml. of five per cent sodium bicarbonate in distilled water. In ten cases the dissolved preparation was added to 200 ml. of physiological saline which was injected by slow intravenous drip over a period of 30 or 60 minutes. In three instances, the hormone was diluted to 1,000 or 1,500 ml. and injected over a period of 16 to 24 hours. The solution prepared in this manner was colorless and opalescent and did not produce either subjective or objective reactions in our volunteers, except for mild anorexia after 12 hours of infusion in one case.

In the short-term experiments, blood samples were drawn immediately before the beginning of the injection and as it ended. Additional samples were drawn at hourly intervals up to four hours after the beginning of the experiment. When prolonged infusions were used, samples were drawn immediately before starting the experiment, before each meal, as the injection was completed, and 24 hours after the beginning of the injection. The blood was deproteinated immediately by the appropriate method, and analyses were performed within 36 hours. Glucose was determined by the iodometric titration method of Somogyi,[42] blood ketones by the method of Greenberg and Lester,[22] slightly modified, α-amino nitrogen according to the method of Frame and Russell,[15, 40] and inorganic phosphate by the technique of Fiske and Subbarow.[14]

In some cases attempts were made to show an effect of the growth hormone on the glucose or insulin tolerance tests. These tests were performed 24 hours before and 24 hours after the start of the growth hormone infusion. Insulin tolerance was tested by the intravenous injection of 0.1 unit of crystalline insulin per kilogram of body weight. Glucose tolerance was measured after the intravenous injection of 25 grams of glucose over a period of about one minute. Both tests were followed for about an hour.

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*Kindly supplied by Dr. L. Mitchell, Frank W. Horner, Ltd., Montreal, Canada, as Lot No. 20-1-2-J-51.

TABLE 1. EFFECT OF INTRAVENOUS GROWTH HORMONE ON BLOOD GLUCOSE AND KETONE BODY CONCENTRATION

Name	Sex	Age	Wt. kg.	Dose mg./kg.	Duration of infusion mins.	Glucose concentration, mg./100 ml. blood — Minutes after starting infusion						Total ketone bodies, mg./100 ml. blood — Minutes after starting infusion					
						0	30	60	120	180	240	0	30	60	120	180	240
SALINE CONTROLS																	
WMU	F	32	88	0	30	97	80	92	80	81	73	5.5	5.0	5.1	5.3	5.0	5.4
ARW	F	38	61	0	30	93	93	84	91	88	82	4.5	5.6	4.5	4.4	4.3	4.3
NB	F	34	53	0	30	77	82	75	75	69	71	*					
EXPERIMENTAL SUBJECTS																	
RLB	F	30	62	1	30	96	100	89	98	87	88	3.1	4.4	7.1	8.7	12.4	8.6
ED	M	33	55	1	60	100		90	99	93	92	2.9		4.6	8.9	11.9	11.9
HM	M	52	46	1	60	82		84	89	82	91	7.3		8.5	7.8	7.0	8.6
JP	M	24	58	1	60	73		73	78	77	78	1.3		1.2	1.5	2.1	2.3
WM	M	21	66	3	60	110		95	98	97	86	2.5		5.2	4.2	7.4	5.0
GB	M	34	70	3	60	90		96	98	96	115						
HMP	M	23	79	3	60	86		86	91	88	88	1.5		1.8	3.1	4.0	4.7

* Technically unsatisfactory because patient had received para-amino hippurate immediately before these observations.

TABLE 2. EFFECT OF INTRAVENOUS GROWTH HORMONE ON PLASMA AMINO ACIDS AND SERUM PHOSPHATE

Name	Dose mg./kg.	Duration of infusion mins.	α-amino acids, mg./100 ml. plasma — Minutes after starting infusion						Inorganic phosphate, mg./100 ml. serum — Minutes after starting infusion					
			0	30	60	120	180	240	0	30	60	120	180	240
SALINE CONTROLS														
WMU	0	30	11.8	9.6	9.2	8.9	7.3	7.1	4.3	4.2	4.3	4.0	4.1	4.0
ARW	0	30	11.0	12.5	9.1	11.7	8.2	7.5	3.4	3.4	3.3	3.4	3.5	3.4
NB	0	30	8.0	6.9	8.4	6.8	7.5	6.8	3.3	3.3	3.4	3.5	3.5	3.6
EXPERIMENTAL SUBJECTS														
RLB	1	30	7.5	10.8	10.7	7.2	8.2	11.2	3.5	3.2	3.2	3.1	3.4	3.5
ED	1	60	7.2		7.0	6.4	6.7	7.1	3.4		3.1	3.2	3.6	3.6
HW	1	60	7.8		6.1	9.1	7.9	6.8	3.8		3.5	3.6	4.0	3.7
JP	1	60	6.5			8.2	7.6	6.2	3.3		3.5	3.4	3.4	3.8
WM	3	60	7.6		7.0	8.4	6.6	5.6	4.0		3.9	3.9	3.8	3.8
GB	3	60	9.1		8.8	8.2	9.0	8.5	4.0		3.6	3.6	3.6	3.8
HMP	3	60	4.9		4.3	5.0	4.8	4.3	4.8		3.2	3.1	3.3	3.5

RESULTS

The experimental results of the acute injections are set forth in Tables 1 and 2. When given over a period of 30 to 60 minutes, one to three mg./kg. of growth hormone failed to affect the concentrations of glucose, α-amino nitrogen, or phosphate in any consistent fashion. The effect of growth hormone on these blood constituents was not different from the effect of saline.

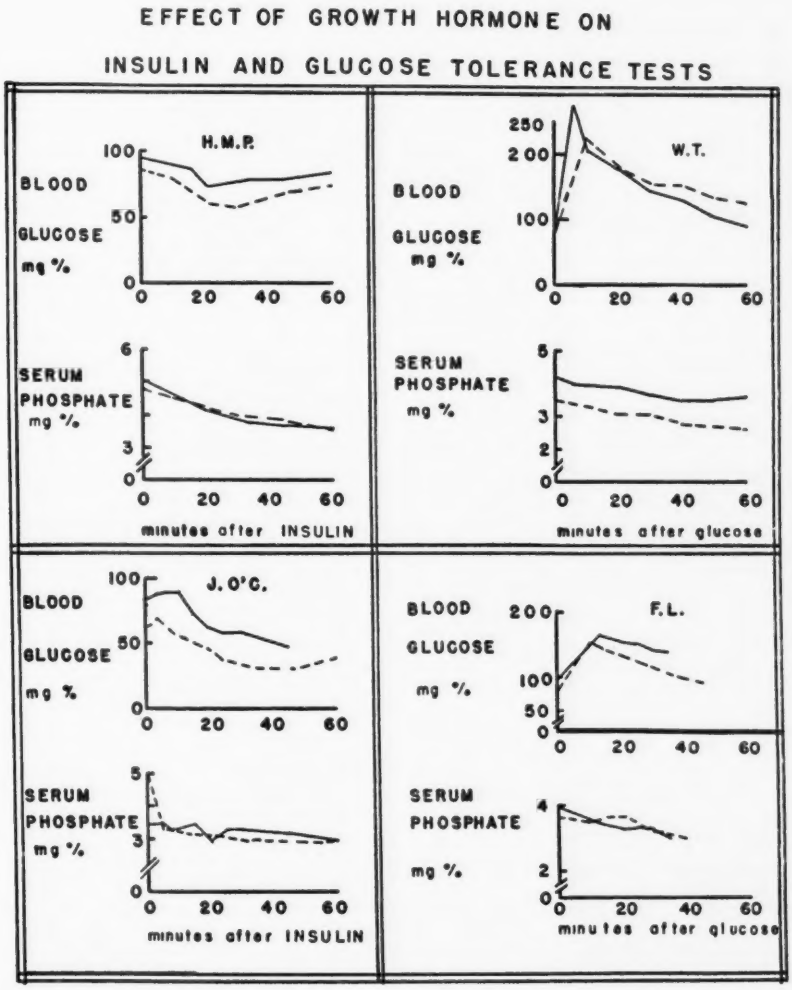

FIG. 1. The effects of intravenously administered purified anterior pituitary growth hormone on the glucose and insulin tolerance. The broken line represents the test before the hormone was given, and the solid line is the curve obtained 24 hours after starting the hormone injection.

TABLE 3. EFFECT OF PROLONGED INTRAVENOUS GROWTH HORMONE ADMINISTRATION ON BLOOD GLUCOSE, KETONES, α-AMINO ACIDS, AND INORGANIC PHOSPHATE

						Glucose concentration mg./100 ml. blood					*Ketone bodies mg./100 ml. blood*					*α-amino nitrogen mg./100 ml. plasma*					*Inorganic phosphate mg./100 ml. serum*				
						Hours after starting infusion					*Hours after starting infusion*					*Hours after starting infusion*					*Hours after starting infusion*				
Name	*Sex*	*Age*	*Wt. kg.*	*Dose mg./kg.*	*Duration of infusion hrs.*	*0*	*4*	*8*	*16*	*24*	*0*	*4*	*8*	*16*	*24*	*0*	*4*	*8*	*16*	*24*	*0*	*4*	*8*	*16*	*24*
JO'C	M	68	61	2.4	16	78	109	119	114	84	1.7	4.4	1.9	..	2.2	7.0	6.9	8.0	8.1	5.5	4.0	3.9	3.6	3.6	3.4
WT	M	25	64	4.7	17	81	96	100	104	82	1.4	1.2	1.2	1.6	10.3	6.1	5.9	4.6	5.5	6.6	3.7	3.4	3.2	3.7	4.2
FL	M	21	76	2.0*	24	79	79	101	119	99	0.4	1.0	1.1	0.8	0.8						3.4	3.4	3.1	3.7	4.0

* In this instance, a Raben-Westermeyer growth hormone preparation[37] supplied by the Horner Company, Ltd., was used.

Growth hormone consistently caused a rise of the blood ketone body concentration, whereas in the two control subjects where this analysis was performed no change of blood ketones occurred. Observations of hematocrits and plasma proteins during the growth hormone injection showed that no significant hemodilution had occurred.

TABLE 4. BLOOD GLUCOSE CONCENTRATION OF NORMAL PERSONS NOT SUBJECT TO ENDOCRINE MANIPULATIONS

	Blood glucose concentration mg./100 ml.		
Name	*Pre-breakfast*	*Pre-supper*	*Change*
JW	63	71	+8
HB	67	70	+3
FW	68	67	—1
WG	61	66	+5
AK	65	82	+17
WW	73	88	+15
AF	74	54	—20
RK	78	51	—27
PW	54	70	+16
GR	60	59	—1
AD	83	70	—13
CM	114	65	—49
JW	117	57	—60
			—8.2 ±6.8*

* Mean ± standard error

During the course of the prolonged intravenous infusions of growth hormone, there was a consistent rise of blood glucose concentration (Table 3). This rise is higher than is seen in normal individuals[44] (Table 4). Moreover, in two of the three cases, the postabsorptive blood glucose concentration 24 hours after starting the injection was significantly higher than the pre-injection concentration. Although there was some rise of blood ketone bodies, this was less pronounced than in the subjects who received comparable doses of hormone in a much shorter period. The changes of α-amino nitrogen are difficult to evaluate, since the plasma concentration of this group of substances is reduced by insulin or glucose, and raised by feeding

protein, so that any type of change might occur during the course of the day as a result of feeding. The fall of inorganic phosphate probably has a similar explanation.

Following the administration of growth hormone there was no convincing change of the shape of the glucose or insulin tolerance curves, although the fasting levels after the hormone tended to be higher, so that the curves were sometimes parallel rather than superimposed (Fig. 1). In one case (F.L.) the glucose tolerance appeared to have been somewhat impaired by growth hormone, but this was difficult to interpret, since technical difficulties made it necessary to reduce the amount injected to only 17.5 gm. and to shorten the time of observation. There was no change in the response of inorganic phosphate to insulin or glucose after the administration of growth hormone.

DISCUSSION

Previous attempts to demonstrate an effect of growth hormone in human beings have usually depended on studies of the nitrogen balance carried on over a long period of time. The doses of hormone were frequently rather small, and the preparations were often somewhat toxic. Under these circumstances it is not surprising that most investigators have failed to find any of the anticipated metabolic effects.[2, 10, 13, 29, 33] In a few cases brief reports have appeared claiming the production of a positive nitrogen balance with various growth preparations, but unfortunately these experiments have not yet been published in sufficient detail to permit evaluation of the data.[8, 37]

In two recent studies, however, definite effects of growth hormone in human beings appear to have been well documented. Carballeira, Elrick, and Browne[7] showed that the blood amino acid concentration fell faster after the intravenous injection of amino acids if the subjects had received growth hormone. Moreover, less amino acid nitrogen appeared in the urine after growth hormone treatment. These data were interpreted as indicating that growth hormone promoted the storage of amino acids in the tissues. The differences reported are small, but they are consistent and probably significant. These authors also reported a rise of blood glucose and ketone bodies after the injection of growth hormone; however, since they never injected the hormone without also giving amino acids, it is difficult to determine the significance of these changes.

Kinsell *et al.*[25] have recently reported that the daily intravenous injection of 100 mg. of growth hormone over a 24-hour period for six days in a diabetic patient on a constant dose of insulin resulted in an increased excretion of glucose in the urine. There was also a rise of ketone bodies, but no consistent change of blood glucose occurred.

The present studies show that the acute intravenous injection of large amounts of the hormone regularly produces ketosis, but has no other detectable effects. When comparable amounts of the hormone are injected slowly over 16 to 24 hours, the production of ketosis is not marked, but a definite increase of blood glucose concentration does occur. This effect does not appear to be reflected in a change of glucose or insulin tolerance.

The pattern of metabolism induced by growth hormone in experimental animals has been well established. Protein is spared from conversion to carbohydrate or fat, or oxidation for energy, and is deposited as new protoplasm.[49] Carbohydrate is likewise spared, both as glycogen,[35] and by virtue of the reduced carbohydrate metabolism, resulting, in its most florid form, in diabetes mellitus.[24, 38, 49] Although the glycogenic effect may be partially antagonized by the release of glucagon, as postulated by Bornstein *et al.*,[4] Young,[49] and Foa *et al.*,[15] and the diabetogenic effect combatted by the release of insulin,[5, 6, 34] these adjustments fail to prevent the development of a depressed rate of carbohydrate utilization.[35]

If protein and carbohydrate become less readily available as energy sources, an increase in fat catabolism must make up the deficit. It is therefore not surprising that growth hormone has been found to cause a mobilization of tissue lipids to the liver[20, 43, 45] via the blood,[5, 43] presumably as a preliminary to increased fat oxidation. As the breakdown of fat increases, ketosis occurs, probably because the load of two-carbon fragments accumulating in the liver exceeds the capacity of the hepatic enzyme systems to oxidize them directly.

It is of interest that ketosis was produced so easily in our subjects, whereas other changes such as alterations of the plasma α-amino nitrogen, blood glucose, or serum phosphate could not be demonstrated acutely. At least two possible explanations may be considered for this discrepancy. The dose may have been too small to produce the nitrogen or carbohydrate effects, or the time of observation may have been too short to permit them to appear. In dogs, ketosis and hyperlipemia occur within a few hours after the administration of the hormone, whereas insulin or glucose tolerance alterations require a much longer period of treatment.[27] Perhaps the same is true of man. In either case, whether because of too little hormone or too little time, it is difficult to escape the conclusion that the shift to a high-fat metabolic pattern is the earliest observed change produced by the hormone. If this is the case, the emphasis on carbohydrate and protein in the preceding discussion may have been incorrect. Instead of considering the ketosis a result of changes of protein and carbohydrate metabolism, it might be more nearly correct to view the shift to a high-fat metabolic substrate as

the basic alteration which precedes and facilitates the more spectacular protein and carbohydrate effects. The observations of Greenbaum[19] that the hormone-induced growth ceases when body fat depots are exhausted supports this viewpoint.

This interpretation is apparently in conflict with the observations that the lipid-mobilizing ("adipokinetic") action of pituitary extracts is not necessarily associated with the growth hormone fraction.[28, 30, 39] It is possible, however, that the fat-mobilizing activity of the pituitary may reside in several different fractions. In any case, it is difficult to see how a material with the metabolic properties ascribed to the purified preparations of growth hormone now available could fail to exert a fat-catabolism-promoting effect.

SUMMARY

The intravenous injection of purified crystalline growth hormone in doses of one to five mg./kg. over periods of 30 minutes to 24 hours failed to alter the plasma amino acid concentration, the serum phosphate, or the insulin or glucose tolerance of normal human volunteers. When the hormone was given rapidly, ketosis developed, but when it was given slowly the ketogenic effect was less marked and a rise of blood glucose occurred.

Since ketosis appeared earlier and more consistently than any other change, and since alterations of fat metabolism occur in animals before changes in carbohydrate or protein metabolism can be demonstrated, it is suggested that the basic effect of growth hormone may be to promote the use of fat as the major metabolic substrate. This would reduce the demand for protein and carbohydrate as sources of energy and facilitate their storage as protoplasm and glycogen, respectively.

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SOME EFFECTS OF THE PERIPHERAL FIELD ON THE CYTOCHEMICAL DIFFERENTIATION OF NEURONS*

Knowledge of the mechanisms regulating the sequence of changes whereby neuroblasts differentiate into neurons is fundamental to an understanding of how reflex arcs and higher association pathways become organized. The manner and extent to which the development of neurons is regulated by intra-cellular, presumably genetic, mechanisms and through the interaction between cells, either those of the peripheral field or other neurocellular elements, remain largely unknown. Observations on the normal sequential changes in form[33] or chemistry[20, 27] of these cells have not provided information pertaining to the mechanisms regulating the changes described.

However, many experiments based on the enumeration of cells[2, 3, 11, 25, 35] show the number of motor neurons ultimately present in the spinal cord of the chick is roughly proportional to the volume of the peripheral field. Further, by correlating the form, spatial arrangement, and sequence of occurrence of unipolar and bipolar neuroblasts, one of the investigators[2] arrived at the suggestion that the peripheral field regulates the final number of neurons present in the motor horn by a process of selective induction of indifferent cells to differentiate into neurons. Unipolar neuroblasts in the motor area became bipolar about the time that their axons reached the uninnervated periphery and, as these neuroblasts became bipolar, those indifferent cells medially adjacent to the now bipolar cells began to develop axons. Accordingly, a reduction of the volume of the peripheral field (by limb bud removal) terminates the process of induction earlier by limiting axon outgrowth, and fewer neuroblasts are called upon to differentiate. Additional support for the induction hypothesis has been presented by Mottet.[31]

Our earlier experiments,[2, 31] though they indicate the progressive changes in the form of neuroblasts that are induced by the peripheral field, provide no evidence regarding the mechanisms responsible for these changes; that is to say, they give no evidence of the dynamics of cell metabolism associated

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with the morphological changes. The importance of these metabolic activities appears, in some cases at least, to be reflected in the chemistry of the cells. For example, in sea urchin eggs[7] and in Drosophilia[14] the mitotic cells contain a high concentration of desoxyribonucleic acid (DRN) in their nuclei and a low concentration of ribonucleic acid (RNA) in their cytoplasm, whereas in comparable inter-mitotic or differentiated cells the relative concentrations are reversed. By means of ultraviolet absorption spectroscopy, Hydén[27] has shown that as mantle cells differentiate into motor neurons in the rabbit fetal spinal cord there is a progressive increase of RNA and a decrease of DRN. This led Hydén to the conclusion that nucleic acids are important intermediaries in the synthesis of neuron cytoplasm. Studying the re-synthesis of axoplasm in regenerating adult primate motor neurons, Bodian and Mellors[6] found an inverse relationship between the concentration of RNA and acid phosphatase enzyme (AP).

Since one aspect of the differentiation of neuroblasts into neurons in terms of form is the increase of cytoplasmic volume, and in view of the projected rôle of nucleic acids and acid phosphatase in the synthesis of cytoplasm, one approach to the analysis of the mechanisms regulating neuron differentiation would be to determine whether or not the changes in concentration of the aforementioned substances are regulated by extracellular (peripheral field) or intracellular forces.

MATERIALS AND METHODS

To demonstrate whether or not the nucleic acids and acid phosphatase activity are affected by the peripheral field we removed the periphery before the neuroblasts had visible signs of differentiation. This was done by removing the right posterior limb bud from chick embryos at 60 to 70 hours' incubation by means of the operative technique described previously.[31] By reducing the volume of the peripheral field unilaterally and thereby affecting the differentiation of neuroblasts homolaterally, the contralateral half of the spinal cord in each tissue section served as the control specimen. Having the control in each tissue section permits the observation of minute changes in cellular chemical reactivity because the possibility of erroneous results due to unequal exposure (in time and concentration) to reagents is thereby completely obviated.

The 70 embryos prepared were killed at daily intervals from four to eight days' incubation and at variable intervals from nine to eighteen days (Table 1). All specimens were placed in fixatives appropriate for the cytochemical methods employed within thirty seconds after interruption of blood supply. From the four- to eight-day embryos the posterior end was dissected by merely transecting the lumbar region, whereas only the posterior portion of the spinal cord and adjacent tissues was removed from the eight- to eighteen-day embryos to facilitate rapid fixation. Serial sections were prepared except in the AP series, and all sections were cut at 10μ.

Nucleic acids. The demonstration of RNA activity by the Nissl stain[5, 18] as modified by Putt[32] was discrete following fixation in 10% formalin for 24 hours. DRN was

visualized by the Feulgen method described by Bensley and Bensley[4] with fixation for 24 hours in a saturated aqueous solution of mercuric chloride containing 2% acetic acid.

Acid phosphatase. The demonstration of AP enzyme activity was achieved by extensively modifying the technique simultaneously developed by Gomori[23] and Takamatsu.[28] The procedure follows:

Acetone (fixation and infiltration)	24 hours
Acetone-acetylcellulose	24 "
Benzene	1 hour
Paraffin (two changes)	1 "

TABLE 1. LIST OF MATERIALS

	Embryos prepared			
		DRN		
Days' incubation	*RNA*	*4" HCl*	*20" HCl*	*AP*
4	2	3	2	9
5	2	..	6	8
6	2	3	3	7
7	2	..	4	3
8	3	3	..	..
9	1	..	..	1
10	1	1	..	..
11	1	..	..	..
15	1	..	..	..
18	1	..	..	1
Totals	16	25		29

Because AP is thermolabile at temperatures above 50° C., a low melting point paraffin (±43° C.) of the following formula was used:

43-45° C. m.p. paraffin	250 grams
Stearic acid	80 "
58° C. m.p. paraffin	50 "
Beeswax	10 "

Neither heat nor albumin was used to mount the section on slides. The ribbon of sections was simply pressed firmly onto the slides then placed in xylol and 100% alcohol for one minute each. To prevent the sections from coming off the slides they were then placed in an alcohol-ether-celloidin mixture. The tissues were next hydrated. Following this the sections were incubated twelve hours at 37.5° C. in the substrate solution described by Gomori.

Results of this technique permit consistently good differentiation eliminating the "unexplained" negatives that occur with Moog's[30] method. Recent improvements of the technique by Goetsch and Reynolds[22] permit more accurate localization of the sites of AP activity within various regions of individual cells.

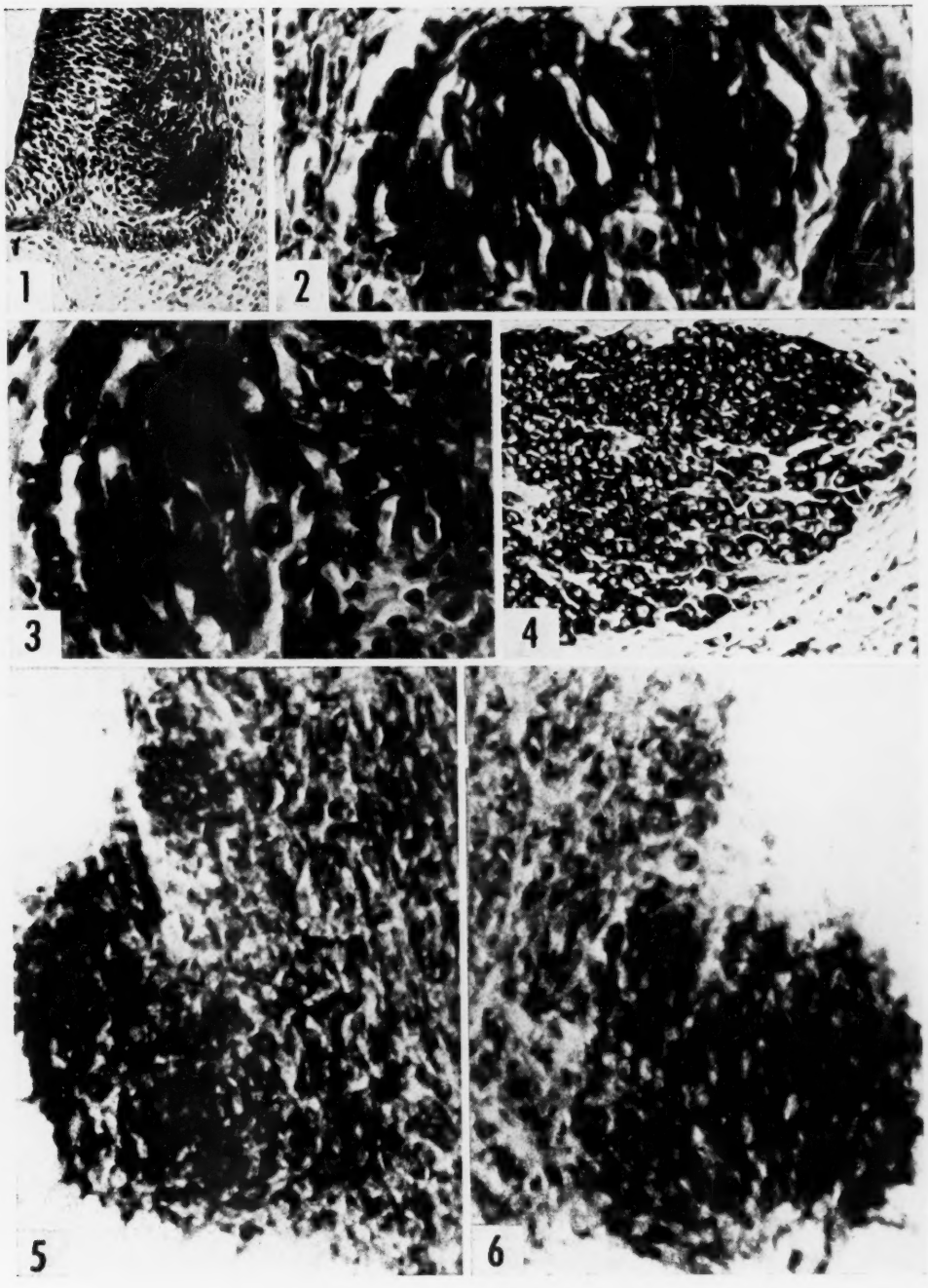
No attempt was made to interpret the results of the techniques employed in terms of absolute quantity or total activity. The conclusions drawn were based on a comparison of relative activity in cells differentiating into neurons with that of indifferent neuroblasts within the same tissue section. The half of the spinal cord contralateral to the affected side of each tissue section served as the control.

OBSERVATIONS

Acid phosphatase. The AP activity was greater in the cells of the spinal cord and ganglia than in the surrounding mesenchyme or epithelium of the nine embryos killed at four days' incubation. However, the renal tubules were stained more intensely than the spinal cord. Within the cord itself the cytoplasm of the indifferent mantle cells appeared homogeneous light brown. The activity in the nuclei of these cells appeared to be much greater, for they stained dark brown to black (Figs. 1, 2, 3). By contrast, those cells within the mantle that were differentiating into unipolar and bipolar neuroblasts, as judged by the intensity of the staining, appeared to have had a greatly increased cytoplasmic AP activity, equaling that of the nuclei. These cells are in the motor area and lateral surfaces of the medullary tube. Similarly, cells differentiating into sensory neurons in the spinal ganglia had an intense cytoplasmic AP activity. Here the cells with increased activity were located in the ventrolateral portion of the ganglia. Axon processes of all differentiating neuroblasts had a moderately intense AP reaction, though less than in the perikarya.

The number of differentiating neuroblasts was comparable in both the affected and control sides in eight of the four-day embryos. In these embryos, the volume of the periphery removed was not sufficient to limit the peripheralward growth of nerve fibers during the period of life. However, from embryo AP 11, a more extensive removal was achieved and the number of cells having an increased AP activity in the motor area of the affected side was less than in the control side. Those cells not induced to differentiate remained indistinguishable from indifferent mantle cells.

In the eight embryos killed at five days' incubation the disparity in the number of differentiating motor cells in the control and affected sides was readily observed on inspection. On the affected side the few motor neuroblasts present were located at the lateral surface of the area near the marginal layer. The area occupied by motor neurons on the control side extended farther medially toward the ependyma. Bilaterally, the cells differentiating into neurons were similar in appearance, with nuclei and cytoplasm having an intense AP reaction. Indifferent cells in the mantle had dark nuclei and light-brown cytoplasm. In the affected motor horn those cells on its medial aspect that were not induced to differentiate remained



FIGS. 1-6

1. Left basal plate of embryo AP11. Four days' incubation. x225. Note increased acid phosphatase in those cells differentiating into neurons.

2. Control motor horn of embryo AP5. Six days' incubation. x600. Numerous motor neuroblasts have an elevated acid phosphatase (compare with mantle cells, same section).

3. Contralateral motor horn of same section as Figure 2. x600. Few motor neuroblasts are present. No evidence of degeneration.

4. Spinal ganglion of embryo N8. Nine days' incubation. x500. Ventrolaterally a few large sensory neurons remain. For explanation see text.

5. Motor horn of embryo N13. Five days' incubation. x600. No degenerating cells. Few motor neuroblasts have an elevated RNA.

6. Control motor horn of embryo N13. x600. Numerous motor neuroblasts are present.

indistinguishable (in terms of AP activity) from other indifferent mantle cells.

The difference in the number of differentiating motor neuroblasts between the control and affected sides of the spinal cord was more obvious in the ten embryos aged six and seven days (Figs. 2, 3). The differentiating neuroblasts, both motor and sensory, appeared black, whereas the indifferent cells remained light brown in color. In not one of these embryos did we observe evidence of regressive cellular changes, either in the spinal ganglia or motor horn. If the regressive changes in differentiating cells are similar to those observed in mature neurons, one would expect to find an elevated AP activity in the degenerating cells.

Ribonucleic acid. Sixteen embryos were studied to determine the differences in the RNA content of the differentiating and indifferent neuroblasts. In the four-day embryos nerve fibers extended equidistant from the cord toward the limb on the left and into the axial mesenchyme on the affected side. The area occupied by motor neuroblasts in the cord and spinal ganglia were of comparable size on the two sides. That the differentiating motor and sensory neuroblasts had a more intense RNA response than did the indifferent cells was clearly visible in the four-day specimens. Their cytoplasm was uniformly dark blue with a clear nucleus and a dark-blue nucleolus. Axons had a very slight RNA response. Those cells in the spinal ganglia that stained intensely were located in the ventrolateral region (Fig. 4).

On the affected side of the specimens aged five through eighteen days, the increased cytoplasmic RNA activity occurred in fewer cells than on the control side (Figs. 5, 6). Those cells on the medial aspect of the motor area that were not induced to differentiate into neurons remained indistinguishable from indifferent mantle cells. Further, we saw no evidence of regressive changes.

Desoxyribonucleic acid. The Feulgen reaction was comparable in all indifferent mantle cells in the four-day embryos. Their cytoplasm, which had no DRN, appeared clear, but the nuclei were a uniform mauve color with interspersed chromatin particles appearing dark violet. Those cells of the ependymal layer that were in the process of mitosis had chromosomes colored dark violet. Neuroblasts which were differentiating into motor and sensory neurons had a less intense Feulgen response than the indifferent mantle cells.

The diminution of the Feulgen reaction was more evident in the five- through ten-day embryos. When the volume of periphery removed had been

sufficient to limit the peripheralward growth of nerve fibers, the number of cells in the motor horn or spinal ganglia having a diminished DRN reaction was less than on the control side. These cells were comparable in form and location to those having an increased RNA and AP response. Cells which were not induced to differentiate into neuroblasts had a Feulgen reaction comparable to the normal indifferent cells.

DISCUSSION

The validity of the cytochemical techniques for demonstrating nucleic acid and phosphatase enzyme activity has been subjected to careful scrutiny and analysis since their inception; therefore, to present here a detailed evaluation of the evidence would be superfluous. A reader can refer to the voluminous reviews of the Feulgen reaction.[18, 19, 26, 29] Also, criticisms of its specificity by the Stedemans,[36] Choudhuri,[15] and Carr[12] have been analyzed by Serra,[34] Caspersson,[13] Stowell,[37] and Brachet.[9] Similarly, the specificity of the Nissl technique for demonstrating RNA has been shown by Caspersson and Schultz,[14] Brachet,[8] Gersh and Bodian,[21] and Kauffmann,[25] by a variety of methods. Since the independent and simultaneous development of the technique for demonstrating phosphatase activity cytochemically,[24, 38] the various steps of the procedure have been critically analyzed by several investigators.[17, 22, 24, 30, 39]

By correlating cellular content with cell activity Brachet[7] obtained cytochemical evidence that sea urchin eggs decrease in cytoplasmic RNA during cleavage. A concomitant increase in DRN in the chromosomes was also observed. This relationship was further studied by Caspersson and Schultz[14] who used ultraviolet absorption spectroscopy to compare nucleic acid concentration in rapidly dividing cells with their differentiated homologue. For these studies the rapidly growing onion root tip cells were compared with the basal cells, and the gut cells of larval Drosophilia were compared with imaginal disc resting cells. In both cases, the rapidly dividing cells had a lower concentration of RNA than the quiescent ones. Also employing ultraviolet absorption spectroscopy, Hydén[27] has shown in the rabbit fetus that as mantle cells differentiate into motor neurons, there is a progressive increase in RNA and a decrease in DRN.

The experiments on motor and sensory neuron differentiation in chick embryos herein reported are in agreement with those of Hydén on the rabbit fetus. In addition, we have demonstrated that in the motor cells the changes in concentration of these substances (also AP) are primarily controlled by the peripheral field. Furthermore, the cytochemical differentiation

of these cells occurs concomitantly with the morphological, namely, the increase of cytoplasmic volume by axon and dendrite growth.

Though our observations are in agreement with those of Hydén, they are at variance with those of Bodian and Mellors,[8] and Bueker, Solnitsky, and Meyers[11] on the re-synthesis of axoplasm in regenerating adult motor neurons. They observed an inverse relationship between the concentration of RNA and AP in pathologically modified neurons (axon amputation). While regressive changes were in progress, RNA was reduced in concentration, whereas AP increased above normal levels. Following this, during the regenerative phase, the RNA increased to the normal level and the AP decreased toward normal.

We interpret the incongruity of cytochemical response in *regenerating* neurons as compared with *differentiating* neuroblasts to be a cytochemical expression of a fundamental difference between differentiation and regeneration. The latter is a response to an injury to the integrity of a neuron, whereas the former is the progressive response of neuroblasts to changes in their environment, resulting in differentiation. Presumably the geneplasm establishes the capacity to produce RNA, DRN, and AP, but the time and degree of expression of these substances are dependent on the environment of the cell (and therefore the nature of environmental modification).

In many cases of neoplastic modification the AP content of central nervous system tumor cells[39] varies with the degree of anaplasia. In astrocytomas the nuclei of the tumor cells stain deeply, whereas their processes stain moderately. Glioblastomas are similar; however, in the medulloblastoma studied the nuclei of the medulloblasts stained deeply while their cytoplasm remained unstained. Even though the small number of tumors of each type studied negates the reliability of interpretation, there appears to be a general correlation between the degree of anaplasia and the cytoplasmic development and less AP. This is comparable to our observations on the cytoplasmic AP content during various stages of neuroblast differentiation.

That nucleic acids and acid phosphatase are in some manner related to the synthesis of cytoplasm seems to be a valid conclusion. The experiments herein described were designed to study the mechanism regulating the cellular concentration of these substances, and thereby the synthesis of cytoplasm. Having observed that those cells that are not induced to differentiate into neurons remain identical with the normally indifferent cells, it follows that synthesis of cytoplasm during neuroblast differentiation occurs as a result of an interaction between tissues—cells of the medullary tube with

those of the mesenchyme. These results, besides showing the concentration of nucleic acids and acid phosphatase to be affected by the peripheral field, provide further support of the previously advanced hypothesis[2, 31] that the periphery controls the number of motor and sensory neurons by selective induction of indifferent cells to differentiate. Thus it may be inferred that the periphery induces a fundamental shift in the metabolism of some of the indifferent mantle cells from the reproduction of nucleoplasm to the synthesis of cytoplasm.

SUMMARY AND CONCLUSIONS

That neuron differentiation in chick embryo spinal cord and ganglia is regulated by selective induction of indifferent cells has been demonstrated by cytochemical means. From 70 embryos the right posterior limb bud and adjacent axial tissue (the periphery) were removed before three days' incubation. The contralateral (left) side was the control. Employing Feulgen, Nissl, and Gomori reactions, the desoxyribonucleic acid, ribonucleic acid, and acid phosphatase enzyme, respectively, were studied in embryos from four to fifteen days' incubation. In the controls the nuclear DRN was diminished in cells differentiating into neurons. Concomitantly, the cytoplasmic RNA and AP reactions became more intense. These cytochemical changes paralleled the morphological. On the affected side the anterior horn and spinal ganglion cells that normally differentiate into motor and sensory neurons remained cytochemically indistinguishable from the indifferent cells. These results show the concentration of nucleic acids and acid phosphatase to be affected by the peripheral field and support the previously advanced hypothesis that the periphery controls the number of motor and sensory neurons formed by inducing indifferent cells to differentiate into neurons.

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DIVERTICULUM OF THE ESOPHAGUS*

INTRODUCTION

This presentation is an attempt to summarize the available information concerning esophageal diverticulum; the pathogenesis and anatomy of its various types; its symptomatology, treatment, and particularly the complications which can occur either spontaneously or as the result of surgery. The case experience with this entity at the Grace-New Haven Hospital (University service) during the period 1942-1953 will be discussed in addition.

The earliest case reported was that of Ludlow in 1764.[2] Zenker[3] in 1877 described 27 autopsied cases with their associated symptomatology and anatomical findings; the pharyngo-esophageal pulsion diverticulum is sometimes known as Zenker's diverticulum.

Esophageal diverticula are classified into three groups which differ not only in anatomical location but also in pathogenesis. The first, and by far the commonest, type is the pharyngo-esophageal, located just above the junction of the hypopharynx and esophagus proper. A second or so-called traction type of diverticulum occurs in the middle portion of the esophagus in relation to the tracheal bifurcation. Finally, a very rare third type is seen in the distal esophagus just above the diaphragm.

Rokitansky[1] in 1840 first attempted to classify esophageal diverticula according to the pathogenetic mechanism. One group, the so-called pulsion type, he considered as arising because of pressures developing within the esophageal lumen; the second, or traction type, because of forces exerted on the esophageal wall from without. This over-simplified concept has been generally accepted even to the present day.

ANATOMY AND PATHOGENESIS

Pharyngo-esophageal diverticulum. Diverticula of this type originate in the posterior midline of the hypopharynx at the pharyngeal "dimple," a

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triangle bounded above by the inferior constrictor muscles and below by the cricopharyngeus muscle. Rarely it originates between fibers of the cricopharyngeus muscle, or below in an area bounded by the cricopharyngeus and the longitudinal muscle bands of the esophagus.[6,7] In extremely rare cases a diverticulum may have its origin laterally between fibers of the constrictor pharyngeus inferior.[5,8] One case of anteriorly placed diverticulum has been described with the orifice located immediately below the arytenoid cartilages.[9] This position is reminiscent of the ventral air bladder in certain fishes (Dipneusti).

As the pulsion and pharyngo-esophageal type of diverticulum develops, its sac usually presents toward the left side of the neck. The wall is composed of mucosa, submucosa, and fibrous tissue of variable thickness. A complete muscular coat is not ordinarily present, but thinned out fibers of the pharyngeal musculature envelop the neck or insert on the proximal portion.[5,6,10,11,12] The majority therefore are not true diverticula but are rather herniations of the inner mucosal and submucosal layers.

Albrecht[6] in 1885 compared these sacs with those occurring normally in certain higher animals such as the pig, camel, and elephant. He also pointed out the possible relationship of this type to the position of the dorsal swim bladder in most fishes. If, however, these pharyngo-esophageal diverticula are congenital in origin, it is strange that no well-authenticated case in the newborn or very young child has been described. King[13] observed none below the age of 15, thus lending strong support to the theory of an acquired lesion. The same writer attributed the genesis to a thinning of the inferior constrictor muscle from repeated pressure exerted by the lower border of the cricoid cartilage against the anterior surface of the cervical vertebrae.

Jackson and Shallow,[14] Davis,[15] and Lahey[10] emphasized the fact that the upper esophageal sphincteric mechanism (the cricopharyngeus muscle) is normally in a state of tonic closure. Jackson postulated that an incoördination of neuromuscular activity during the act of swallowing resulted in an abnormally elevated intraluminal pressure and a tendency to herniation of mucosa through the site of natural muscular weakness (the pharyngeal "dimple"). No proof of such neuromuscular incoördination has been presented.

Harrington[6] believed that the primary etiological factor is a muscular deficiency at the site of herniation. Liebow[5] suggested that a secondary traction factor might be superimposed on a pulsion effect as a result of organizing peridiverticulitis.

Once a diverticulum is formed, it tends to increase in size for several reasons. First, there is no fascial barrier to limit extension into the superior mediastinum, and the negative mediastinal pressure tends to accelerate this process; second, food and secretions enter the sac before the esophagus proper. The esophageal orifice is eventually reduced to an eccentrically placed, crescentic slit[4] difficult to visualize.

The very rare lateral type of diverticulum may well be a manifestation of persistent branchial cleft in its proximal portion.[21]

Traction diverticulum. Traction diverticulum is observed almost exclusively in the middle third of the esophagus. The organization of mediastinal infectious lesions, and especially tuberculous lymph nodes, causes fibrotic traction on the adherent esophageal wall. The resultant diverticulum is usually not larger than one or two centimeters; rarely it reaches an enormous size (see Fig. 1). The orifice of the sac is ordinarily at a lower level than its fundus, so that retention of food, secretions, and foreign bodies is minimal and growth of the sac is not rapid. In a rare case pulsion effects contribute to a later enlargement. As anticipated by the mechanism of their origin, this traction type of diverticulum shows a wall composed of mucosa, submucosa, and muscularis.

Benign mural tumors such as myoma have been known to exert traction effects during their growth.[16, 17] The resultant diverticulum can, of course, occur anywhere in the esophagus.

Epiphrenic diverticulum. Epiphrenic diverticulum is the rarest of the three main types. Little is known of its etiology. Raven[18] suggested that some form of distal esophageal obstruction results in increased intraluminal pressures and herniation through areas weakened by local esophagitis. Kay[19] presented a similar theory, but excluded the need for esophagitis being present. The demonstrated association of cardiospasm with about half the cases of distal epiphrenic diverticulum tends to support the obstructive hypothesis, but cardiospasm can be an effect rather than a cause.[20] As in the case of pharyngo-esophageal diverticulum, the wall of the sac is usually composed of mucosa, submucosa, and an outer fibrous covering of variable thickness.

SYMPTOMS AND PHYSICAL SIGNS

Pharyngo-esophageal diverticulum. Dysphagia of insidious onset is usually a first symptom of pharyngo-esophageal diverticulum. Sometimes, however, the symptoms are sudden in onset and rapidly progressive in severity.[8] In addition to dysphagia there may be regurgitation of undigested food, even many hours after its ingestion, particularly when the patient lies

down or bends over. Gurgling or croaking noises are heard during swallowing of liquids, and this may be the earliest recorded symptom. Sialorrhea, cough, dyspnea, and hoarseness can be present. Fetor oris results from bacterial decomposition of the sac contents. Eventually, complete obstruction of the esophagus can result with all of its sequelae.

On physical examination one may observe at times an asymmetry of the neck, with a palpable fullness beneath the lower portion of the sternomastoid muscle. An actual mass is seldom felt unless the diverticulum is complicated by carcinoma, granuloma, or abscess. Pressure on the side of the neck sometimes produces an audible gurgle or initiates an episode of regurgitation. Auscultation of the neck during the swallowing of water may demonstrate a succession of splashing sounds.

Traction diverticulum. Symptoms caused by this type are infrequent, most cases being discovered as incidental findings at autopsy or during barium study of the gastro-intestinal tract. When dysphagia is present, it can usually be explained on the basis of a fibrosing mediastinal process, benign stricture, or carcinoma of the esophagus. However, Wallace[22] stated that over a third of his patients with radiological evidence of traction diverticula were symptomatic as a direct result of the lesion. Symptoms included substernal pain, burning and heaviness, as well as dysphagia. The reported cases of diverticula caused by myomata were not known to have symptoms.

Positive physical findings are not present unless complications occur.

Epiphrenic diverticula. Because of the high incidence of associated cardiospasm, symptoms directly due to epiphrenic diverticula are often difficult to evaluate. They vary from mild dyspepsia to dysphagia, substernal or epigastric pain, regurgitation, eructation, and even vomiting after meals. Again there are no pathognomonic physical findings.

TREATMENT

Von Bergmann[23] performed the first successful single-stage pharyngoesophageal diverticulectomy in 1891. This procedure was associated with a high incidence of septic mediastinitis in the earlier cases because of leakage through the esophageal suture line. It remained for Goldmann[24] to develop an operation which was relatively safer. In 1909 he reported a two-stage procedure; the first consisted of simply freeing up the diverticulum and suspending it by suturing it to the cervical fascia. In the second stage the diverticulum was excised through an area safely walled off by adhesions. In 1921 Bevan[11] reported a method for invaginating smaller sacs, thus obviating an esophageal suture line.

Although Lahey[10] actively supported the two-stage procedure, the one-stage operation has been preferred in many clinics of this country and abroad.[5, 6, 14, 15, 25] The lower incidence of recurrence, the absence of significant sepsis under antibiotic protection, the shorter and more comfortable hospitalization all speak in favor of the one-stage procedure. Details of this operative procedure may be found elsewhere.[5]

Epiphrenic diverticula, when symptomatic, may be treated by one of two methods. In extremely poor risk patients without cardiospasm, transthoracic diverticulopexy (suspension) is most expeditious. Otherwise, a direct trans-

TABLE 1. COMPLICATIONS OF PHARYNGO-ESOPHAGEAL DIVERTICULUM

Preoperative
1. Foreign body perforation or ulceration
2. Pleuropulmonary infection
3. Obstruction and its sequelae
4. Malignancy
Operative and Postoperative
1. Injury to recurrent laryngeal nerve
2. Sudden emptying of sac contents
3. Fistula
4. Stricture
5. Recurrence

thoracic excision of the lesion is the treatment of choice. If the offending diverticulum is associated with intractable cardiospasm, esophagogastrectomy should be considered.

COMPLICATIONS OF DIVERTICULUM

Complications of pharyngo-esophageal diverticulum may be divided into three groups; preoperative or spontaneous, operative, and postoperative (see Table 1).

Spontaneous perforation is usually the result of the ingestion of a foreign body such as chicken bone. This may result in an acute para-esophageal phlegmon, which can extend rapidly into the mediastinum. Sometimes a local abscess or a solid granuloma develops, which may be palpated as a deeply seated, slightly tender cervical mass.

The potential dangers of esophagoscopy must be emphasized. If rupture of a sac is recognized during or immediately after esophagoscopy, the treatment of choice is an emergency cervical exploration under local anesthesia with excision of the sac and primary closure of the esophagus. If several

hours or days have passed since perforation and signs of mediastinitis are evident, cervical and superior mediastinal drainage should be performed as for any upper esophageal perforation. One should not rely entirely on antibiotics and medical measures in this circumstance. After several months, when the inflammatory reaction has cleared, the sac may be excised safely. Consideration should be given, however, to a first-stage suspension procedure at the time of cervical drainage, if the patient has been troubled by severe dysphagia, if the local infection is not too far advanced, and if the sac is easily mobilized.

Single-stage resections may be safely performed in the presence of local chronic abscesses or granulomas. Unless these are located in the neck of the sac, there should be no difficulty with the esophageal suture line.

Pleuro-pulmonary infection is usually the result of regurgitation and aspiration of sac contents, especially when reclining and during sleep. Acute exacerbations of chronic organizing pneumonitis, pulmonary abscess, and even empyema may complicate the clinical picture. Bronchiectasis may eventuate. Advanced and neglected cases present with inanition, hypochromic anemia, hypoproteinemia, and vitamin deficiencies. These must be combatted by vigorous parenteral therapy. Gastrostomy or jejunostomy should be avoided if possible. It is more satisfactory to prepare the patient, at least partially, with parenteral therapy and then to carry out a first-stage suspension of the diverticulum under local anesthesia. When the patient has restored his proper nutritional status, a second-stage extirpation of the sac is performed. This should not be delayed indefinitely, since there is a slight risk of malignant degeneration. As in this series, others have reported the occurrence of carcinoma with preëxistent Zenker's diverticula. They are usually of the well-differentiated epidermoid variety.[26, 27, 28]

Hoarseness, while most often due to local mechanical factors, may actually be the result of recurrent laryngeal paralysis.[9] This is caused either by involvement of the nerve in an inflammatory process or operative injury to the recurrent laryngeal nerve. The latter can be avoided by tracing the nerve upward from the inferior thyroid pole and protecting it during the further dissection. Hoarseness may be permanent, but it is usually temporary because the nerve has been traumatized by crushing or traction rather than by transection. Heavy retraction on the carotid vessels should be avoided because of the danger of cerebral vascular accidents.

Sudden emptying of the sac contents during surgery and resultant aspiration can be minimized by preliminary lavage of larger sacs. This is done by the insertion of a catheter and irrigation of the diverticulum with warm saline or water twice a day for several days prior to surgery. The

FIG. 1. Barium study reveals a large diverticulum of the traction variety in the mid-esophagus. The patient, a 78-year-old male, died of unrelated causes. Autopsy revealed the diverticulum to be adherent to the right lower lobe and adjacent mediastinal structures by dense fibrous adhesions.

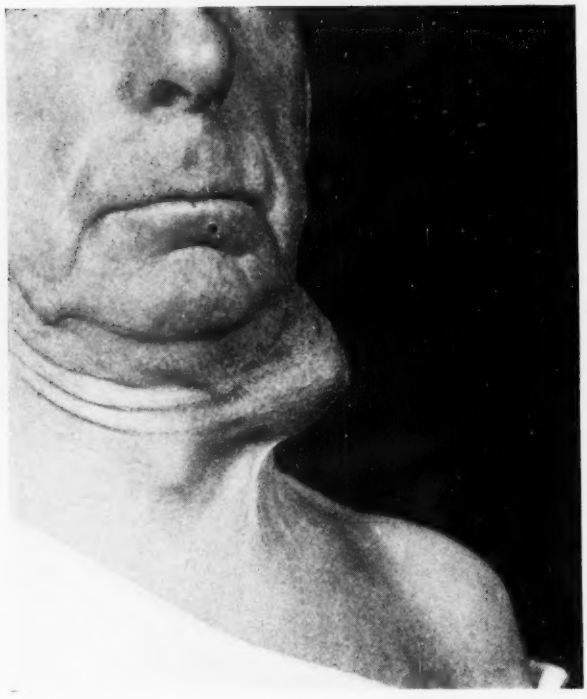

FIG. 2A. Photograph of an 82-year-old male who underwent suspension of a diverticulum five years previously. It shows a mass under the scar, subsequently proven to be epidermoid carcinoma in the diverticulum.

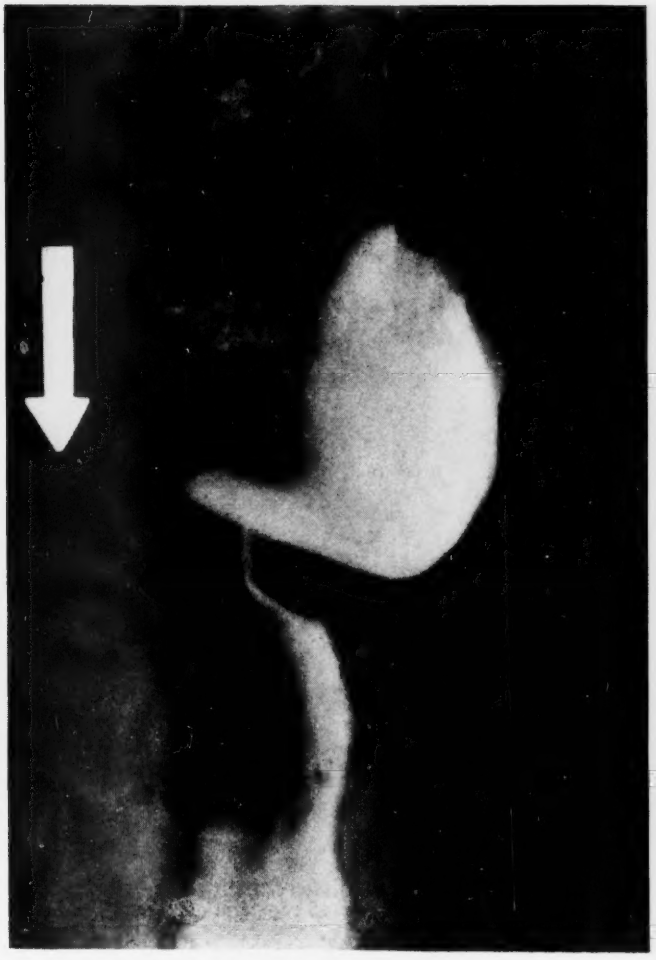

FIG. 2B. Roentgenogram of same case reveals an irregular filling defect at the fundus of the diverticular sac.

FIG. 3. Esophagogram taken ten months after an initial single-stage diverticulectomy in a 48-year-old male. Symptoms necessitated re-operation. There has been no evidence of recurrence since the second operation.

patient's diet should be liquid during this period. Preoperative antibiotics are given, especially when pulmonary infection is present. If the patient is coöperative, local anesthesia can be employed. If general anesthesia is necessary, it should be administered through a cuffed endotracheal tube.

Postoperative fistula is now rarely seen. Its incidence may be decreased by meticulous technique and a two-layer closure of the esophagus with fine silk or cotton. Slip drains should never be placed directly on the line of closure. One-stage resections in malnourished patients with severe hypoproteinemia should not be performed. Fistula formation is a serious threat only when it occurs in the first day or so after surgery. Then diffuse mediastinitis can result, but this is generally mild with adequate wound drainage and proper antibiotic therapy. Late fistula formation results in delayed wound healing and requires multiple dressings, but it is almost always self-limited. Two sequelae may occur. One is recurrent deep cervical abscess, the other stricture of the esophagus.

Stricture of the esophagus may result also because of too extensive resection at the diverticular neck. To prevent this, guide sutures should be used to mark the junction of the diverticulum with the esophagus above and below the neck of the sac. The danger of excessive resection is especially marked in very small diverticula, and this is one reason for conservative management of early cases. Inadequate excision of a diverticulum on the contrary may result in a recurrence. Every case should be evaluated after surgery by repeat barium esophagogram before the patient is discharged from the hospital or from follow-up. A definite recurrence with return of symptoms necessitates another single-stage operative resection (Fig. 3).

At the site of a traction diverticulum the esophagus may perforate into the mediastinum, into the trachea, or a major bronchus.[29] Severe mediastinitis in this location requires a posterior mediastinotomy. Tracheo-esophageal fistula acquired in this manner must be treated by surgical closure and the interposition of a pleural or fascial flap.

Epiphrenic diverticulum may be complicated by peptic ulceration, but foreign bodies are probably the greatest offenders in the production of ulcerations within these rare lesions.[30] The treatment of this complication is the same as for the diverticulum itself.

ANALYSIS OF NEW HAVEN HOSPITAL CASES

Between the years 1942 and 1953, 15 cases of pharyngo-esophageal diverticulum were treated surgically on the University service of the Grace-New Haven Hospital. Twelve were males and three, females. This distribution is similar to Starck's[4] reported ratio of approximately three to one. The

mean age at the time of surgical admission was 56 years, the extremes being 41 and 75.

The average duration of symptoms prior to surgical admission was three years. Fourteen of the 15 operative cases complained of regurgitation as a primary symptom. Dysphagia was present in 12, cough in five, and gurgling sounds during deglutition in four. Two patients had noted excessive salivation; a like number were dyspnoeic, probably as a result of tracheal compression by large diverticula. One patient complained of hoarseness.

Preoperative complications in one advanced case included cachexia, aspiration pneumonitis, and empyema. Another patient aspirated a small amount of barium into the trachea during a radioscopy, but without any detectable sequel.

Definite physical findings attributable to the diverticulum were noted in over half the cases. The clinical impression was confirmed by barium swallow, esophagoscopy, or both. Perforation of the sac during the esophagoscopy occurred once; the procedure was being carried out by a well-trained otolaryngologist. A successful issue ensued after immediate primary resection of the ruptured diverticulum.

The surgical approach was through the left side of the neck in 12 of the 15 cases, through the right in the remaining three. Twelve of the patients had only cervical block and local procaine. Primary excision of the sac was carried out in 13 despite the presence of a recent instrumental perforation in one case (as mentioned above), and a small mural abscess in another. Local wound infection occurred in two cases and was associated with the development of stricture at the operative site in one. This patient required temporary gastrostomy and dilatations, but was asymptomatic one year after operation. Transient recurrent nerve paralysis was observed three times with spontaneous recovery in each.

Two patients of the 15 were subjected only to the conservative procedure of suspension of the diverticulum. One was the patient with extreme cachexia, empyema, and hypoproteinemia. Having been entirely relieved of his dysphagia, he decided against further surgery and returned five years later with a palpable and enlarging mass in the region of the cervical scar. This proved to be an epidermoid carcinoma in the diverticulum and was treated at age 82 by block excision (Fig. 2).

One patient with roentgenographic evidence of incomplete excision of the diverticulum continued to have mild symptoms with occasional regurgitation. A second case presented a definite and large recurrence one year after primary excision and required secondary diverticulectomy which has apparently been curative (Fig. 3).

There were no deaths in the series of operative cases. During the same period six additional cases of pharyngo-esophageal diverticulum were observed, but not treated surgically. The mean age in this group was 72 years. Three had no symptoms and one was the victim of recurrent cerebrovascullar accidents. The disposition of the remaining two is unknown.

Traction diverticulum. During the years under consideration 21 cases of traction diverticulum were revealed by barium studies. The mean age was 66 with extremes of 44 and 81 years. There were 13 males and 8 females. Only two of the 24 had symptoms definitely referrable to the lesion; one of these had an associated stricture in the distal third of the esophagus. Three patients had radiological evidence of subcarinal or parahilar calcified lymph nodes. Five other patients had concomitant diverticular formations, two in the duodenum and three in the colon.

Epiphrenic diverticulum. Four cases of epiphrenic diverticulum were diagnosed between 1942 and 1953. One required surgery because of epigastric pain, weight loss, and dysphagia. This case has already been recorded in detail elsewhere.[20]

SUMMARY

The recognized types of esophageal diverticulum have been considered from the standpoint of anatomy, pathogenesis, symptoms, physical signs, and treatment. Twenty-one cases of pharyngo-esophageal, 21 cases of mid-esophageal traction, and four cases of epiphrenic diverticulum have been studied. The result of surgery (diverticulectomy) in 16 of these patients has been analyzed. The complications which may precede or follow upon surgery for this interesting and relatively rare condition have been discussed in some detail with suggestions for decreasing their incidence and for their successful management.

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THE EFFECT OF COLLAGENASE ON HUMAN SKIN COLLAGEN. COMPARISON OF DIFFERENT AGE-GROUPS AND OF CASES WITH AND WITHOUT "COLLAGEN DISEASE"

That rheumatic fever generally attacks children and rheumatoid arthritis usually occurs in adults may be due to an alteration in the target organ (collagen plus ground substance) with age. Perhaps young, growing collagen is more susceptible to the stimuli producing rheumatic fever, whilst the more metabolically inert adult collagen responds with rheumatoid change to factors as yet unknown. One approach to the problem would be the estimation of the rate of hydrolysis of collagen by the enzyme collagenase. This paper describes the results of such hydrolysis on the skin collagen of 75 cases representing all age groups and including 13 patients with so-called "collagen disease."

PREPARATION OF SUBSTRATE

1. *Skin collagen.* (a) *Unextracted.* In an effort to keep the condition of the substrate as near as possible to that found *in vivo,* unfixed autopsy dermis or Achilles tendon was homogenized in demineralized water in a Waring blendor in the cold room for 15 minutes. The final temperature of the suspension was 30° C. or less in each case. After centrifugation for 20 minutes at 2,000 RPM at 4° C., the sediment was dried in a vacuum. This simple method had to be reluctantly abandoned for the following reasons: (1) *The presence of impurities* as demonstrated by (a) increased amount of amorphous material and a "coating" over the fibers when viewed under the electron microscope, (b) the significantly higher tyrosine readings described below, (c) the cloudy supernate in the "test" tubes following manual shaking at the end of incubation as compared with clear fluid in the controls and in "test" tubes containing extracted substrate; (2) *the smaller hydrolysis* evidenced by comparison of macroscopic digestion and total nitrogen determinations on unextracted and extracted samples of the same substrate (Table 1).

(b) *Extracted.* Neuman[12, 13] has described a method of preparation which he states does not denature or alter the properties of collagen, involving prolonged extraction at 4° C. employing 10% NaCl, N/15 Na_2HPO_4,

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TABLE 1. COMPARISON OF DIFFERENT INCUBATION PERIODS AND ENZYME CONCENTRATIONS ON EXTRACTED AND UNEXTRACTED COLLAGEN OBTAINED FROM THE SAME 9811 ADULT SKIN (73 YRS.). 25 MG. SUBSTRATE PER TUBE. T — (C + E) = AVERAGE TEST READING MINUS AVERAGE CONTROL READING PLUS ENZYME NITROGEN.

			mg. N/100 mg. dry wt. of collagen					
			Average TEST reading	*Average CONTROL reading*	*Enzyme Nitrogen*	*T — (C + E)*	*mg. N/100 mg. dry wt. solid collagen*	*Taking pure collagen at highest reported total N (18.6 g.%) this corrects for non-protein material in original sample.*
COLLAGENASE 0.1 ml. 0.5% solu. or 0.1 mg. enzyme nitrogen	Incubated 18 hrs. at 37° C.	Extracted skin 9811 (73 yrs.)	14.4	2.5	0.1	11.8	15.0	$\frac{18.6}{15.0} \times 11.8 = 14.6$
		Unextracted " " "	12.4	2.4	0.1	9.9	12.9	$\frac{18.6}{12.9} \times 9.9 = 14.2$
	Incubated 3 hrs. at 37° C.	Extracted " " "	8.77	2.28	0.1	6.39	15.0	$\frac{18.6}{15.0} \times 6.39 = 7.8$
		Unextracted " " "	5.75	1.68	0.1	3.97	12.9	$\frac{18.6}{12.9} \times 3.97 = 5.7$
COLLAGENASE 0.2 ml. 0.5% solu. or 0.2 mg. enzyme nitrogen	Incubated 3 hrs. at 37° C.	Extracted " " "	11.7	1.61	0.2	9.89	15.0	$\frac{18.6}{15.0} \times 9.89 = 12.2$
		Unextracted " " "	8.45	2.34	0.2	5.91	12.9	$\frac{18.6}{12.9} \times 5.91 = 8.6$
	Incubated 6 hrs. at 37° C.	Extracted " " "	12.6	2.05	0.2	10.45	15.0	$\frac{18.6}{15.0} \times 10.45 = 13.0$
		Unextracted " " "	8.8	2.34	0.2	6.26	12.9	$\frac{18.6}{12.9} \times 6.26 = 9.0$

acetone, alcohol, and ether. In this study, a shortened form was utilized with extraction for a minimum of one day with each of these reagents. The collagen was washed once after the salt, and three times after the phosphate, the ether was changed once, the fibers air dried and carefully shredded. Only suitable fibers were used, badly blended small lumps were discarded. Care was taken that comparable surface areas were presented for enzyme action from the different specimens.

2. *Calves tendon.* A sample involving prolonged extraction[12,13] was supplied by Dr. J. D. Ogle.

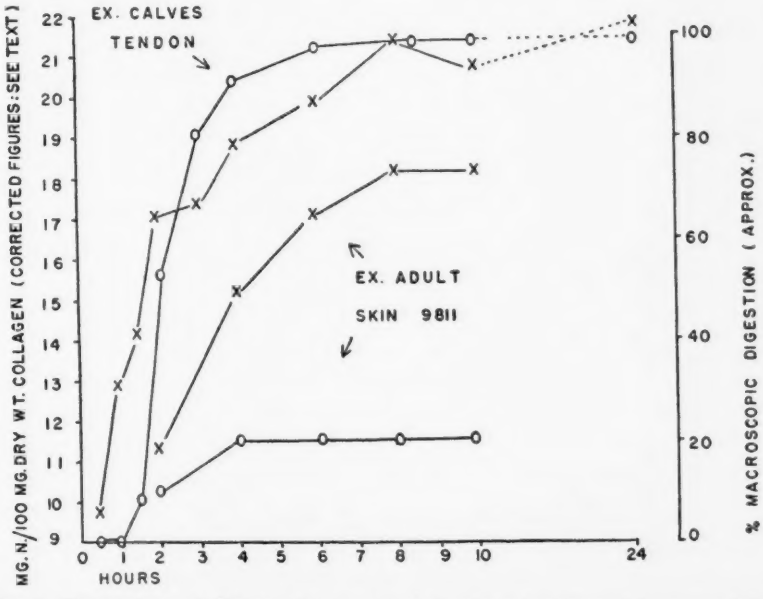

FIG. 1. Comparison of filtrates from extracted calves tendon and human adult skin collagen 9811 (10 mg. dry weight per tube) following action by collagenase (0.1 ml. of a 0.5% solution or 0.1 mg. enzyme nitrogen per tube) x——x = Micro-Kjeldahl estimations; 0——0 = Macroscopic digestion (approx.).

COLLAGENASE

Dr. J. D. MacLennan made available some collagenase obtained from *Cl. histolyticum.* It contains some proteinase, but is one of the purest preparations available at the moment, as a crystalline form has yet to be made. Five milligrams of *chemically collagenase-free proteinase* also supplied by Dr. MacLennan produced only negligible hydrolysis when incubated with 20 mg. of the same adult skin collagen used in the experiments reported in Table 1 and Figure 1 for 20 hours at 37° C. Even if it is

assumed that the collagenase contains as much as 50% proteinase, then the concentration of proteinase used in this experiment represents more than 100,000 times the quantity used per tube throughout this investigation.

DETERMINATION OF OPTIMAL EXPERIMENTAL CONDITIONS

As both skin collagen and collagenase can only be obtained conveniently in small amounts, a procedure had to be found that would give maximum accuracy with a minimum of material. It was important to choose an enzyme concentration and incubation period that would reveal any alteration in *rate* of hydrolysis with age or disease. Lengthy incubation would simply give the *end result* of the enzyme action, which might not show much difference with age, whereas the rapidity with which the reaction occurred could conceivably have been very different. Owing to the possibility of a marked variation occurring in different diseases it was desirable to stop the reaction one-half to two-thirds towards completion to allow for variations in each direction.

It was known that 0.1 ml. of a 0.1% solution of the particular collagenase used in this study would cause complete solution of 25 mg. of extracted calves tendon collagen following incubation at 37° C. for 18 hours in 5 ml. phosphate buffer at pH 7.3 (Dr. J. D. MacLennan, personal communication). Consequently, in preliminary work 25 mg. dry weight of collagen was used in both test and control tubes, and 10 mg. for estimation of the nitrogen content of each individual sample. Even so, allowing for wastage and unextracted fibrous lumps, this required a piece of skin measuring 2½ by 1½ inches, and a larger area from infants and young children where the dermis is very thin. In later work this quantity was reduced to 20 mg. and samples not supplying suitable material were discarded.

Figure 1 and Table 1 show timing experiments on extracted collagen from human skin and calves tendon with two different concentrations of enzyme: 0.1 ml. of an 0.5% solution and 0.2 ml. of an 0.5% solution of collagenase. The tendon collagen hydrolysed more rapidly and completely than adult skin and closely followed the visible digestion. The collagen from adult skin broke down more slowly and showed a macroscopic digestion curve characteristic of collagen from adults, only 10-50% of the solid going into solution. As shown in Table 1, at 18 hours the reaction was complete and there was no difference between extracted and unextracted substrates, whereas at three and six hours there was a significant difference between the two collagens. At three hours, digestion was one-half to two-thirds complete and since this was not appreciably altered at six hours it was concluded, in view of the need to conserve enzyme, that three hours'

incubation with the weaker concentration of collagenase (0.1 ml. of an 0.5% solution or 0.1 mg. enzyme nitrogen per tube) would give the desired effect. This would produce approximately half the soluble nitrogen found at completion, i.e., 18 hours. The decision was confirmed by the level attained at three hours in Figure 1 which showed 60-70% hydrolysis.

METHOD

Unfixed, frozen autopsy and biopsy abdominal skin from the left upper quadrant was thawed, the dermis minutely dissected out and the collagen extracted as described above. Twenty milligrams were incubated with collagenase (0.1 ml. of an 0.5% solution or 0.1 mg. enzyme nitrogen) and 0.05 ml. penicillin and streptomycin mixture in a total volume of 5 ml. phosphate buffer (pH 7.3) for 3 hours at 37° C. Control tubes were treated in the same way, including the antibiotic mixture but without enzyme. After incubation the tubes were inspected macroscopically and the clarity of the supernate and amount and disintegration of the substrate were compared with the control both before and after manual shaking. After filtering through Schleicher and Schnell No. 576 filter paper, 1.0 ml. duplicate aliquots were taken for standard micro-kjeldahl determinations. Preliminary timing tests showed that the nitrogen recovery from the filtrate from calves tendon, human tendon, and human skin was complete in one hour. In addition to the test and control filtrates the nitrogen content of samples of solid collagen from each specimen was determined without using collagenase. Ten milligrams dry weight of collagen carefully selected to be representative of that used in the "test" tube was digested for 1½ hours and distilled for 15 minutes. In a few cases 5 mg. was used owing to insufficient material.

CALCULATION OF RESULTS

The average mg. of nitrogen per ml. of "test" filtrates x the reaction volume $\left(\frac{5}{1}\right) \times \frac{100}{20}$ = mg. N/100 mg. dry weight of collagen. This test reading minus the control reading (obtained in a similar manner) minus the enzyme nitrogen (0.1 mg.) was the amount of nitrogen actually derived from the hydrolysis of the collagen sample. However, a correction was necessary owing to the inevitable variability of the substrate (e.g. hair and fat content), the specimens being obtained from skin of different age groups suffering from a variety of diseases. This was partially overcome by multiplying the amount of nitrogen obtained from the equivalent of 100 mg. dry weight of substrate by an experimentally determined correction factor. The nitrogen content of various animal collagens in the literature ranges from 17.4—18.6 g%.[13, 8] If 18.6% were taken as representative of "pure" (ox hide) collagen[8] and used as the numerator, and the nitrogen content of solid samples of each individual specimen of substrate used as the denominator, multiplication of readings by this factor would render the results compar-

able. For example, if the solid substrate gave 15 mg. N. per 100 mg. dry weight and the filtrate yielded 9.9 mg. N. per 100 mg. dry weight of the same individual specimen, then $\frac{18.6}{15.0} \times 9.9 = 12.2$. The figures used in the graph and tables were corrected in this way. Of course, this "factor" only corrects for non-protein non-collagenous material in the original substrate such as fat, and the micro-kjeldahl determinations pick up nitrogen from hair follicles, etc., but such an error would be small and fairly constant throughout the study.

RESULTS

Macroscopic changes. Correlation between visible digestion of the substrate and the amount of soluble nitrogen in the filtrate was imperfect. As a general rule there was an obvious increased disappearance in infants and young children as compared with adults, but in some cases with no apparent digestion there was evidence of appreciable hydrolysis in the filtrate. This underlines the difficulty of assessing the volume of solid collagen present when the fibers are hydrated and dispersed. One notable exception was a man aged 24 years dying of subacute bacterial endocarditis superimposed on rheumatic heart disease: the collagen was 80-85% digested in the three hours which is equivalent to that found in infants under one year.

The clarity of the supernate in the control tubes was unaltered after incubation both before and after shaking. Nearly all the "test" tubes also remained clear, but a few exhibited very slight cloudiness after shaking. In contrast, earlier experiments using unextracted collagen showed marked cloudiness of the test supernate and disintegration of the substrate following shaking. As mentioned above, this was believed to indicate the presence of extraneous non-collagenous protein in the collagen, and preparation by this (simpler) method was abandoned.

Chemical results. In the 75 cases tested, Table 2 shows there is a decrease in hydrolysis with increase in age. Infants produced nearly twice as much, and children aged one to ten years half again as much soluble nitrogen in a given time as adults over the age of 30. However, there is quite a wide scatter in each decade. The cases of collagen disease (excluding dermatomyositis) gave higher average readings than those dying from other causes, but this difference was not significant. The only exception was one 13-year-old child diagnosed clinically as rheumatoid arthritis who gave a very low reading. One very acute case of dermatomyositis with widespread histological necrosis gave extremely low readings for the age group and showed no macroscopic evidence of substrate digestion. Rash-bearing skin

from the upper chest gave lower readings than apparently normal abdominal skin, but both samples exhibited negligible hydrolysis.

There were certain individuals dying from diverse other diseases whose collagen gave results different from the average for their particular age group. A single case of carcinoma of the lung (Table 2), aged 52 years, gave a very high reading only equalled by infants under one year and the 24-year-old man dying from subacute bacterial endocarditis previously mentioned. The patient had no history or clinical evidence of rheumatic fever or rheumatoid arthritis, but had been given intravenous nitrogen mustard daily for the four days before his death. Each of five cases of diabetes mellitus gave low readings well below the average for their age groups. One seven-year-old child with acute leukaemia and one woman aged 68 with cirrhosis of the liver, oesophageal varices, pancreatitis, and a recent splenorenal shunt operation also gave low readings. One substrate did not extract well, being pale yellow in color due to the presence of fat. This gave a falsely low reading presumed due to the "protective envelope" of fat around the fibers preventing enzyme action. Care was taken that subsequent specimens were adequately extracted.

DISCUSSION

The increased collagen breakdown by collagenase in the younger age groups and the decreased hydrolysis in adults (Table 2) is compatible with previous work on collagen metabolism in animals. Neuberger, Perrone, and Slack[11] showed that the radioactive glycine turnover in rat tendon was very low in adult but more active in young, growing animals. They concluded that adult rat tendon collagen is "metabolically almost completely inert." Further experiments[10] showed that rat skin readings closely followed those of tendon, the figures for young, rapidly growing animals being three to four times that of adults. Subsequent studies on collagen formation in rabbit skin by Harkness and Neuberger[8] showed a more rapid turnover of radioactive pro-collagen in young rabbits that decreased with age. Using glycine-N^{15} Robertson[14] presented evidence suggesting that the nitrogen turnover in skin collagen of guinea pigs took place without appreciable breakdown of the macromolecule. He stated that the concept of the dynamic state of the body proteins, demonstrated for many proteins, would not seem to apply to collagen. Although Table 2 shows a decrease in hydrolysis with age, the levels are more or less constant after growth has ceased. The increased digestion of skin collagen in babies and children may be due to increased susceptibility of (growing?) collagen and/or increased accessibility to the enzyme of the smaller, finer fibers found in babies.[2] These fibers

TABLE 2. MGN/100 MG. DRY WEIGHT COLLAGEN (CORRECTED FIGURES: SEE TEXT) FROM EXTRACTED ABDOMINAL SKIN COLLAGEN (20 MG. DRY WEIGHT PER TUBE) FOLLOWING INCUBATION WITH COLLAGENASE (0.1 ML. OF 0.5% SOLUTION OR 0.1 MG. ENZYME NITROGEN PER TUBE) FOR THREE HOURS AT 37° C. THIRTEEN CASES OF COLLAGEN DISEASE AND 62 CASES DYING FROM OTHER CAUSES.

	Age in years									
Disease causing death	under 1	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90
Leukaemia		14.2 15.8 13.1 15.8 11.7 1.8	5.4							
Lymphoma		15.0	15.6							
Reticulo-endotheliosis								9.2 9.4		
Congenital heart or congenital defects	15.1 13.6 16.8 14.5 14.0 17.2		6.8							
Erythroblastosis foetalis	15.8 12.6 13.8									
Brain tumor				10.1	10.0	8.2				
Carcinoma							16.2‡ 5.8	8.6		
Adenocarcinoma						10.2	10.7 6.8		5.3§ 7.6 7.6	
Diabetes mellitus					4.8	5.3	6.4 6.6	5.8		
Coronary thrombosis				9.1		9.8		10.3	10.1	
Arteriosclerosis									9.8 8.3	
Hypertension							8.1	9.2		
Nephritis				10.4						
Uraemia						9.2 4.9				

Pancreatitis				9.6						
Cirrhosis of liver								3.8		
Gastroenteritis							7.6			
Perforated peptic ulcer										8.8
Perforated gall bladder										7.0
Ulcerative colitis					10.9					
Pneumonia	16.1	13.6				11.6				
Lung abscess							8.2			
Mental defect: sepsis		7.2								
Accident		10.9								
No. of cases	10	10	3	4	3	7	9	7	6	2
Average reading	14.95	11.61	9.3	9.8	8.5	8.45	ECL=7.5 ICL=8.5	8.0	8.1	7.9
Dermatomyositis		1.2* 0.47†								
Polyarteritis nodosa			12.2							
Diss. lupus erythematosus			10.3 13.2		11.8					
Rheumatoid arthritis			0.36			10.4 8.3	12.2	11.5		
Rheumatic heart disease				16.2	11.2 13.0	11.6				
No. of cases	.....	1	4	1	3	4	1	.....	.....	.....
Average reading	.....	1.2	9.0	16.2	12.0	10.6	11.5	.....	.....	.....

* Dermatomyositis: abdominal skin
† Dermatomyositis: rash-bearing skin
‡ Carcinoma of lung
§ Fatty specimen giving falsely low reading
ECL = Excluding carcinoma of lung
ICL = Including carcinoma of lung

are separated by a larger volume of ground substance than the thicker, closely packed adult fibers.[6]

The fact that collagen from cases of collagen disease gave higher average readings than those dying from other causes, but that this difference was *not* significant, is somewhat surprising. If collagen plus ground substance forms the target organ in so-called "collagen disease," one would expect evidence of increased metabolic activity *in vivo,* i.e., a reversion to the "growing form" required for tissue repair and accompanied by the increased digestibility found in babies and children. However, as mentioned above, the difficulty may be mechanical, the closer-packed adult fibers being less accessible to the enzyme. A more probable explanation is the metabolic inertia of collagen already discussed.

The lack of hydrolysis in the case of dermatomyositis was particularly interesting in view of the work of Holborow and Keech.[9] They found *no* intradermal spreading action with hyaluronidase in three cases of dermatomyositis, whereas spreading occurred in tuberculous, rheumatic, rheumatoid, and normal subjects, and patients suffering from polyarteritis nodosa, erythema nodosum, and disseminated lupus erythematosus. Normal or increased spreading was found in renal and cardiac oedema and in new and old scar tissue. They concluded that the hyaluronic component of the skin in dermatomyositis is either deficient or unresponsive to testicular hyaluronidase. From the single case described above, it would seem that the skin collagen is also altered, rendering it resistant to hydrolysis with collagenase.

A small but constant amount of tyrosine in collagen has been reported by some workers[8] but denied by others.[1] In view of the speed and simplicity of such a test it was considered worthy of trial. Concurrent estimations of tyrosine and total nitrogen were made on the same filtrates in 25 cases, and from timing tests on extracted calves tendon and extracted adult skin. Immediately after incubation with collagenase the filtrates were read in a Beckman spectrophotometer (Model DU) at 280 m μ. Comparison of tyrosine liberation and micro-kjeldahl estimations were without value, there being no difference between one and six or one and ten hours' incubation and no correlation with nitrogen content. Unextracted collagen gave significantly higher optical densities than extracted samples from the same skin, presumed due to the higher tyrosine content of non-collagenous impurities.

Other speedy methods of estimations of protein hydrolysis were considered[4, 5, 7, 15] but all required the assumption that collagen from different individuals would be split in a similar sequence at a similar rate. Since different amino acids give different color intensities, one could not be sure,

when using different substrates, that double the hydrolysis would, for example, double the biuret reading.

It is fully realized that the *in vitro* action of an isolated enzyme on a substrate isolated from the body and removed from its normal metabolic environment is far from ideal. It is to be hoped that experimental conditions more nearly approaching the conditions *in vivo* will soon be evolved.

SUMMARY

The effect of collagenase on human skin collagen was studied in 75 cases representing all age groups, and including thirteen patients with so-called "collagen disease." A constant skin site (upper abdominal) was used and the amount of hydrolysis estimated by micro-kjeldahl determinations. There was a decrease in hydrolysis with increase in age, infants producing nearly twice as much, and children aged one to ten, half again as much soluble nitrogen in a given time as adults over the age of thirty. The cases of collagen disease gave higher average readings than those dying from other causes but this difference was not significant. One case of dermatomyositis exhibited negligible hydrolysis quite unlike the other cases of collagen disease or other samples in the same age group.

ACKNOWLEDGMENTS

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FAILURE OF LIPOID THROMBOPLASTIN TO ACT AS COFACTOR FOR TRYPSIN IN THE ACTIVATION OF PROENZYMES*

Trypsin is known to activate certain proenzymes, including trypsinogen and chymotrypsinogen.[13] It also activates prothrombin.[1, 9] In the case of prothrombin activation, platelets have been shown to complement the effect of trypsin. Small quantities of trypsin and platelets together exert a much greater effect than the sum of their separate effects.[9] Lipoid thromboplastin has been found to complement trypsin in a similar manner.[2, 11]

The mechanism of this complementary effect is unknown. Therefore, it is of interest to inquire whether lipoid thromboplastin functions as a simple cofactor for trypsin in the activation of proenzymes, generally.

MATERIALS AND METHODS

Buffer: 0.9 per cent NaCl; 0.02M veronal buffer, pH 7.4.

Prothrombin preparations: These materials were obtained by modifications of the methods previously described for prothrombin preparation II[10] and prothrombin preparation III.[11] Preparation II was obtained by taking the material left in solution after isoelectric precipitation at pH 4.6 and aging four days at 5° C.; whereas preparation III was derived from the material precipitated at pH 4.6.

Lipoid thromboplastin (lipid): A cephalin-containing fraction from bovine brain, prepared as described previously.[9]

Crystallized trypsin: Supplied by Armour and Co., Chicago. Three hundred mg. of this material, containing about 120 mg. protein (plus 180 mg. magnesium sulfate) was dissolved in 20 ml. 0.0025M HCl and dialyzed against cold 0.0025M HCl.

Crystallized chymotrypsinogen: Prepared by Armour and Co. by a process involving four crystallizations from ammonium sulfate solution followed by two crystallizations from alcohol. It was reported by the manufacturer to contain less than 0.3 per cent ash. One hundred twenty-six mg. was dissolved in 10 ml. 0.0025 M HCl to give the stock solution.

Crystallized trypsinogen: Prepared according to the method of Kunitz and Northrop[8] by Worthington Biochemical Laboratory, Freehold, New Jersey.

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Inhibitor-free trypsinogen: Prepared from crystallized trypsinogen in this laboratory according to the method described by Kunitz[4]; 570 mg. of moist filter-cake were dissolved in 5.7 ml. 0.005M HCl to give the stock solution.

Assay for thrombin: As described.[8]

Rennet activity of chymotrypsin: As described by Northrop, Kunitz, and Herriott.[10]

Proteolytic activity: Casein digestion method of Kunitz.[5]

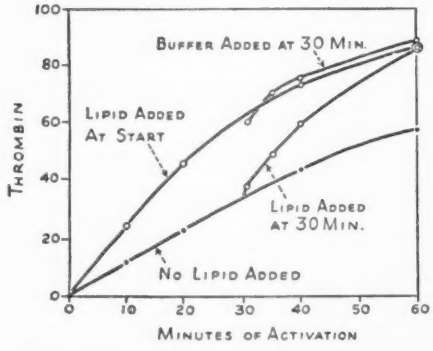

Fig. 1. Activation of prothrombin by trypsin without added lipoid thromboplastin, and with lipoid thromboplastin added at different times. One-tenth ml. prothrombin was mixed with 0.7 ml. buffer, 0.1 ml. of a 1 : 100 dilution of lipoid thromboplastin, 0.1 ml. of a 1 : 1,000 dilution of stock trypsin, and 0.1 ml. $CaCl_2$ 0.0275M. In a parallel experiment, buffer was substituted for lipoid thromboplastin. A separate pair of experiments was set up at the same time and in the same way, except that each contained 0.1 ml. less of buffer. This pair was allowed to incubate for 30 minutes without removal of samples. Then, 0.1 ml. lipoid thromboplastin was added to the mixture which had not received the lipid at the start; and 0.1 ml. buffer was added to the one which had started with added lipid. The activation mixtures contained 0.00055 mg. trypsin per ml. All tests were performed at room temperature.

EXPERIMENTAL

Figure 1 deals with the activation of prothrombin by trypsin. There it is apparent that lipoid thromboplastin, added either at the start or during the activation of prothrombin, increased the rate of thrombin production. When the lipid was added at 30 minutes, the curve of thrombin production did not shift suddenly to the upper curve, but approached it gradually. If the lipid effect had been due to a direct influence on the estimation of thrombin, the curve would have shifted immediately.

These and previous[11] results eliminate the possibility that the lipid effect depends on a spurious increase in thrombin values. It must be concluded that the net production of thrombin is actually faster in the presence of the lipid.

When crystallized chymotrypsinogen was used as the proenzyme, the results were quite different. Even though calcium chloride was included, the lipid had no effect on the activation of chymotrypsinogen by trypsin, as shown in Figure 2. The comparatively large amounts of protein materials used in this experiment were dictated by the standard methods used. If less chymotrypsinogen had been used, the rennet (milk-clotting) activity would have been hard to measure in the early stages of activation.

Consequently, a separate series of experiments was done in which the activation of chymotrypsinogen was followed by estimating its increasing proteolytic activity. Four pairs of tests covered the range of 57 to 456 micrograms of chymotrypsinogen per ml. activation mixture. In each case, the proenzyme was activated by 0.00055 mg. of trypsin per ml. activation mixture, the same concentration as that used for the prothrombin activation of Figure 1. Lipoid thromboplastin was added to one test of each pair. In no case did the lipid make any significant difference in the activation of chymotrypsinogen.

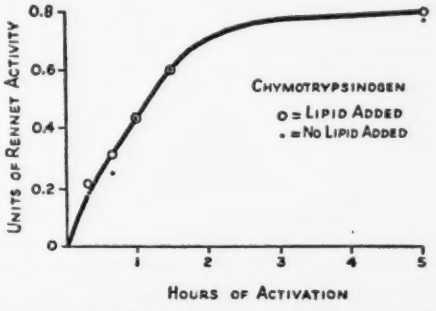

FIG. 2. Activation of crystallized chymotrypsinogen by crystallized trypsin, with and without lipoid thromboplastin. One ml. stock chymotrypsinogen was mixed with 6 ml. buffer, 1 ml. $CaCl_2$ 0.0275M, and 1 ml. of a 1 : 100 dilution of lipoid thromboplastin. Activation was begun by adding 2 ml. of a 1 : 200 dilution of stock trypsin. In the control, 1 ml. buffer was substituted for the thromboplastin. There were 1.1 mg. chymotrypsinogen and 0.0055 mg. trypsin in each ml. activation mixture. The activations were carried out at room temperature; but the serial tests for rennet activity were performed at 35° C.

Certain aspects of trypsinogen activation seemed to offer a more favorable chance of detecting an effect. In the first place, ionic calcium is known to be required for the effect of lipoid thromboplastin[10]; and it is also known to be favorable for the activation of trypsinogen.[7] Secondly, because it is autocatalytic, the activation of trypsinogen is sensitive to a slight, early acceleration. A cofactor, even if it remained effective for only a short time, would shift the sigmoid portion of the curve to an earlier position. It would have an effect similar to that of seeding the reaction with a small amount of trypsin. No such effect is evident in Figure 3.

As these negative findings accumulated, it was necessary repeatedly to verify the activity of the stock thromboplastin. By chance, one recheck was negative—the stock lipid now had little or no effect on the activation of prothrombin by crystallized trypsin. However, further tests soon showed that it was the prothrombin reagent which had changed, rather than the lipid. This was originally encountered with prothrombin preparation I,[10] a material obtained by adsorption with magnesium hydroxide.[8] When fresh, this preparation gave a pair of curves like that of prothrombin preparation III in Figure 4. However, after several days' storage at 5° C. it gave a pair of curves like that of prothrombin preparation II. This difference in

reaction-pattern has been encountered many times since, on several occasions with prothrombin preparations tested simultaneously.

During work on purification of prothrombin, this reaction-pattern has been used as another means of characterizing prothrombin fractions. Various preparations have shown varying degrees of difference in their thrombin production curves, with and without added lipoid thromboplastin.

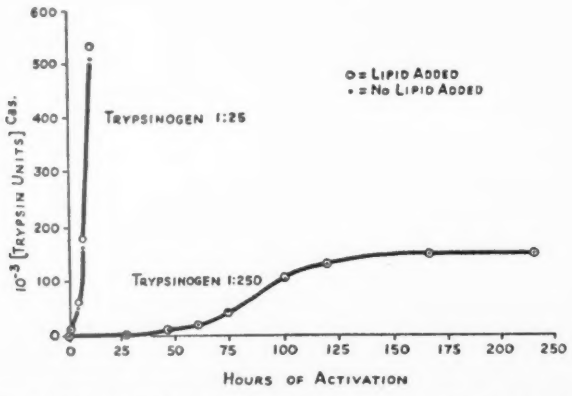

FIG. 3. Autocatalytic activation of crystallized trypsinogen, with and without lipoid thromboplastin. Stock trypsinogen was diluted 1:2.5 and 1:25 with 0.005M HCl; 0.5 ml. diluted trypsinogen was mixed with 3.5 ml. buffer, 0.5 ml. of a 1:100 dilution of lipoid thromboplasti nand 0.5 ml. $CaCl_2$ 0.2M. In the controls, buffer was substituted for thromboplastin. The curves are labelled according to the final dilutions of stock trypsinogen in the activation mixtures. They correspond respectively to trypsinogen concentrations of the order of 0.025 and 0.25 mg. per ml. activation mixture. Activations were carried out at 4-5° C. Serial tests for proteolytic activity were done at 35° C.

DISCUSSION

In the experiments reported, lipoid thromboplastin failed to behave as simple cofactor for trypsin in the activation of chymotrypsinogen, trypsinogen, and prothrombin preparation II. But the lipid *seemed* to behave as cofactor for trypsin in the activation of prothrombin preparation III.

Conceivably, the prothrombin molecules in preparation II may have been different from those in preparation III. Or the difference in behavior may have depended on the impurities in the prothrombin preparations. In the latter case, several mechanisms other than simple cofactor action would be conceivable.[9] The possibility that the lipid takes part in a more complex cofactor effect is not eliminated. And the possibility that the lipid combats an anticoagulant is still open.

Further investigation of these possibilities would be more profitable as the purification of clotting factors progresses. Meanwhile, the difference in

rate of thrombin production, observed with and without lipid, serves as an additional means of characterizing prothrombin fractions. This applies specifically to those cases in which crystallized trypsin is used as the activator and the prothrombin contains little, if any, thrombokinase as a contaminant.

It is of particular interest to note the trend of the evidence concerning the function of lipoid thromboplastin. Howell, himself, did not believe that his

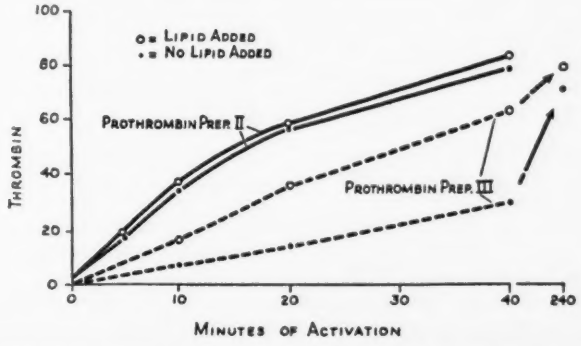

FIG. 4. Activation of two different prothrombin preparations by crystallized trypsin, with and without added lipoid thromboplastin. One-tenth ml. prothrombin preparation III was mixed with 0.7 ml. buffer, 0.1 ml. of a 1:100 dilution of lipoid thromboplastin, 0.1 ml. of a 1:1,000 dilution of stock trypsin and 0.1 ml. $CaCl_2$ 0.0275M. In the control, buffer was substituted for thromboplastin. On a different day, 0.2 ml. prothrombin preparation II was mixed with 0.6 ml. buffer, 0.1 ml. lipoid thromboplastin, 1:100, 0.1 ml. trypsin, 1:1,000 and 0.1 ml. $CaCl_2$ 0.0275M. The control had buffer instead of thromboplastin. All tests were performed at room temperature.

"cephalin" was a direct activator of prothrombin.[8] It has long been known[14] that partially purified prothrombin is activated only very slowly in the presence of calcium and "cephalin." A negative result after an incubation period of 80 minutes has been reported.[9] More recently, it has been possible in this laboratory to obtain a prothrombin preparation which was less than one per cent activated after 28 hours with calcium and lipoid thromboplastin at room temperature.[12] Now an example has been presented (Fig. 4) in which the lipid had very little effect, even as an accessory factor for the production of thrombin.

SUMMARY

In the experiments reported, lipoid thromboplastin failed to function as simple cofactor for trypsin in the activation of crystallized chymotrypsinogen, crystallized trypsinogen, and certain preparations of prothrombin.

With other preparations of prothrombin, the lipid seemed to act as cofactor for trypsin; but this may well have been due to some effect other than simple cofactor action. Since different prothrombin preparations behaved differently in the test system, this system has been used as an additional means of characterizing prothrombin fractions.

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RALPH LINTON 1893-1953

Professor Ralph Linton, Sterling Professor of Anthropology at Yale since 1946 and one of the leading cultural anthropologists of this country, died on 24 December 1953 of a coronary attack. He had been a close friend and a great admirer of the chairman of his department, Wendell Bennett, who was so tragically drowned last September. The premature deaths of these two national leaders who were almost as well known and recognized abroad as at home is a grievous loss to anthropology, to Yale, and to their wide circle of friends.

Dr. Linton was also an authority on mental disease among primitive peoples and it is fortunate that he was spared to give the Thomas William Salmon Memorial Lectures on the 2d, 9th, and 17th of last November on the general subject of "Culture and mental disease." In these lectures Dr. Linton summarized his vast knowledge of a field which has come to be of increasing interest and importance as problems of mental derangement are subjected to closer scrutiny. *The Lancet* of London, with characteristic promptness, published an editorial summary of the lectures in its number for 26 December 1953, and the Editor has graciously permitted *The Yale Journal* to reprint the editorial in its entirety. The extent of the comment is indicative of Linton's stature as an anthropologist and we feel that it is a fitting tribute to the memory of one of the great scholars of our time.

THE CULTURE AND THE BREAKDOWN

In finding expression the troubled mind makes use of the material to hand. Any contemporary event, great or trifling, can be dramatised to make an anxiety dream, any current belief harnessed to a hysterical manifestation. Prof. RALPH LINTON, in the Thomas William Salmon lectures delivered last month to the New York Academy of Medicine, pointed out that in fact the influence of the culture runs through the manifestations of mental disorder in every society. The relation of the individual with his culture is reciprocal: he is shaped by it and he contributes to its shaping. As he grows up, his culture influences him in three ways—by what other people do to him (especially in the way of child care and training), by what they deliberately teach him, and by what he sees of their behaviour to other people. The last is a stronger influence than is often supposed, for the ability of the child to compare the treatment he is getting with that received by others affects his valuation of himself, and helps to determine his relationship with his parents. We are still not sure at what period the culture exercises its maximum influence on the developing personality, though clearly it will be during the early years of life. Prof. LINTON notes, however, that recent research suggests that infantile experiences—toilet training and nursing patterns—are not such important personality determinants as was formerly supposed.

The influence of the individual on his culture is exerted when, as an adult, he is able to accept or reject new ideas. Since all members of a society are exposed to much

the same cultural influences, it is not surprising that certain personality resemblances run through particular societies, though of course there is no general identity of personality structure: all types of personality can be found in any society (provided it is reasonably large), but the frequency with which a given type of personality appears will differ from one society to another. The type of personality occurring most often in a particular society is termed the "basic personality"; and the basic personalities of different societies naturally differ considerably. Except in cultures undergoing rapid change, the basic personality will be congruous with the culture, and those who are happily equipped with it will be able to participate in the culture with a minimum of frustrations and a maximum of rewards. All societies have neurotics, psychotics, and hysterics, recognised by other members of the society as such; but their symptoms differ from society to society in ways which strongly suggest that they have been shaped by cultural influences. Moreover societies differ in the way they deal with people showing these disorders, and some make social use of particular forms of psychic abnormality. The abnormalities themselves, however, seem to crop up in all societies, and as LINTON puts it, "individuals with the constitutional defects responsible for such abnormalities would be abnormal in any society." Probably these defects have a physiological basis. Where the personality of an individual departs widely from the basic personality of his society, tests, history-taking, and analytic studies indicate that his early experience has often been atypical for his society.

It would be interesting, of course, to know something more about psychoses in non-European societies, but the data are scanty. The most useful reports come from Africa, where all types of insanity found in Europeans seem to be present, though the frequencies differ. Thus depressive reactions are exceedingly rare in Africans—and so, indeed, is self-reproach. The African blames his environment rather than himself, and hence his psychotic response is more often paranoid than depressive. It is noteworthy that among delusional psychotics, literates who have had a European education develop symptoms essentially European in type, whereas non-literates show the African type of response. From this it has been argued that Europeanisation, and the resultant cultural stresses, is *not* a significant causal factor in psychosis. Self-reproach and depressive states are rare likewise in Brazilian Negroes. Among these, physical catatonia amounting to waxy flexibility has been seen in patients showing only superficial social withdrawal. Satisfactory cultural explanations of these differences, LINTON says, have not yet been offered. Membership of a tribe, all closely related, is a great support, and may check a patient's impulse towards withdrawal. Again, perhaps the violent emotional expression which these cultures permit makes self-reproach unnecessary. But, though primitive peoples respect psychotics, regarding them with fear and awe, there is no society, Professor LINTON finds, where the genuine psychotic occupies a favoured social position. To exploit the advantages of abnormal behaviour one must know very well what one is about; and the inability to do this is the real test of psychosis. Medicine men and prophets who make a good thing out of their eccentricities do not qualify as insane.

Neuroses seem to be as widely distributed as psychoses, though we have no clue to their relative frequencies among primitive peoples. However, their universality is an argument against FREUD's theory that they originate in sexual repression: for they

Alburtus—Yale News Bureau

Professor Ralph Linton is shown trying a mask on his secretary, Miss Claire Vernick. The Bird Mask, representing the spirit of wind and flight, is made of lightweight wood and is used in religious ceremonies by the Dogong tribe of French Sudan. The photograph was taken in October 1953, shortly after Dr. Linton acquired the headpiece to add to his collection of native masks from all over the world.

appear in societies where expression of the sexual impulse is largely unlimited. It is more probable, LINTON thinks, that they result from the consistent frustration of *any* primary drive. In the Marquesas, for instance, neurotic expressions centre round food rather than sex. In any case there is probably an underlying constitutional factor, for in all societies many people endure experiences which make others neurotic, without becoming neurotic themselves.

New evidence shows that certain types of hysteria have geographical distributions, and that these often cut across racial lines. *Amok* is a form of aggressive suicide practised by males: they attack everyone they meet until they are finally killed; *latah* is a condition of complete submission, in which the patient, when startled, imitates the speech and actions of the first person he (or she) sees. *Amok* is limited to South-east Asia, but *latah* is found in Mongolia, North Africa, and the Near East, and is also a component of Arctic hysteria. Supposed spirit possession is found throughout most of the Old World, but not in Moslem countries, where possession by spirits other than demons is thought to be impossible. Among American Indians *amok, latah,* and spirit possession are almost unknown, though conversion hysterias are common. In Europe and America the pattern of hysterical symptoms has changed in the last few generations. Spirit possession, common in the seventeenth century, has almost disappeared, and so has *grande hystérie.* So, too, has fainting, the hallmark of the real lady; but social distinctions in hysteria are still with us: during the late war conversion hysterias were almost confined to other ranks; officers had nervous breakdowns. LINTON concludes that though a predisposition towards hysteria may be of physiological origin, its manifestations are shaped by the culture to an even higher degree than are those of neurosis and psychosis. The hysterias, of course, bring advantages: "they are designed not only to elicit favourable response from the audience but also to inconvenience and annoy the hysteric's associates," and must be regarded either as an escape mechanism or a response to ego deprivation. It is worth bearing in mind how intolerable must be the situation, how deprived the ego, to drive the patient to such lengths.

Linton had travelled extensively, studying tribal customs in Polynesia, Central Africa, and Madagascar, and had become one of our chief authorities on primitive medicine and the tribal medicine man. During an expedition to Madagascar in 1925-1927 he became so popular with one of the tribes which had just lost its medicine man that his services were enlisted to take the place of the tribe's departed mentor. Realizing that custom among such peoples demanded that all medicines be given with some form of ritual, usually a chant, he taught the tribe to sing "The wearing of the green" each time a quinine pill was administered—this, as he said with a twinkle, to confuse future Irish anthropologists! I am sure that the Yale students who heard Professor Linton's memorable lecture on the medicine man given in our general course on the history of medicine on 21 October 1953 will not soon forget the colorful personality of the lecturer nor what he had to say.

Those of us who so often enjoyed his company at Pierson College of which he was a Fellow will remember Linton as a genial conversationalist

who spoke interestingly and with relevance on many subjects far removed from his sphere of primary concern. He had an outgoing nature which, despite his cardiac difficulties, always sloped toward the sunny side.

Others, better qualified than I, will appraise his many and varied contributions to cultural anthropology. I offer this brief tribute as one who came to know him at Pierson where he was well liked by students both of the College and graduate schools—eloquent testimony of the place he held in their hearts was seen in their attendance in large numbers at the memorial service in Dwight Chapel on 5 January conducted by the Reverend Sidney Lovett and the Reverend Burton A. MacLean.

JOHN F. FULTON

EVENTS

WOODWARD LECTURE

January 13, 1954

THE BIOSYNTHESIS OF GLYCOGEN FROM LACTATE. By H. A. Krebs, University of Sheffield, Sheffield, England.

Previously it has been widely assumed that glycogen biosynthesis is a reversal of glycolysis. Although most of the enzyme catalyzed reactions of glycolysis are indeed readily reversible, there are three which are not. It is at these three points that gluconeogenesis and glucose degradation are catalyzed by a different set of enzymes. The first and second of these steps, which cannot be readily reversed, involve the utilization of adenosine triphosphate for the esterification of hexoses (glucose→glucose-6-phosphate and fructose-6-phosphate→fructose-1,6-diphosphate). The reverse reaction, the dephosphorylation is, therefore, catalyzed by specific phosphatases, which liberate inorganic phosphate from glucose-6-phosphate and from fructose-1,6-diphosphate. The third step which does not proceed readily is the phosphorylation of pyruvate to phosphoenol-pyruvate. Instead, a more complex series of reactions is utilized. Pyruvate is reductively carboxylated in the presence of TPNH and CO_2 to malate. The latter is oxidized to oxaloacetate, which then in the presence of ATP yields phosphoenol-pyruvate and CO_2. In this manner the formation of phosphoenol-pyruvate, which cannot be accomplished readily in a single step, is broken up into three smaller steps. Thus again the pathways of synthesis and breakdown are different from each other. If we write the reactions of synthesis and degradation together: pyruvate→malate→oxaloacetate→phosphoenolpyruvate→pyruvate, we realize that we are dealing with a cyclic process.

E. RACKER

ISAAC RAY LECTURES

October, November, and December 1953

THE PSYCHOLOGY AND PSYCHOPATHOLOGY OF THE CRIMINAL ACT AND PUNISHMENT. By Gregory Zilboorg, New York, New York.

The time has come for law-makers to re-examine the validity of the legal and penal codes in view of society's goals and the findings of psychology and psychiatry in the past fifty years.

Justice Holmes once stated that punishment serves to admonish others not to "go and do likewise," though such behavior may admittedly have been inevitable for the criminal punished. The proper application of law, however, is for justice, not for a mechanical following of the law for the sake of following the law. What is justice applied to one criminal, may, applied to another, verge on criminality. It was the recognition of this which resulted in the woefully inadequate M'Naughten rule. This rule states, in effect, that anyone in a state of insanity, to the extent of not being aware of the nature and quality of his act, is not to be held responsible for his crime.

"Insanity" is a nebulous term, undefined in contemporary medical literature. There is practically no agreement on the medical meaning of this

term nor on the medical interpretation of the phrase "nature and quality" of an act. Even trying to work within the loose connotative framework of the word "sanity" poses a problem, since there is no sharp dividing line between "sanity" and "insanity."

Society desires to get rid of the doer as quickly as possible in order to forget the deed as quickly as possible. But it is the duty of medicine and law to understand the doer in order to help to avoid such deeds in the future. One must separate the moral qualities of the deed from the clinical state of the doer if one is to understand. Otherwise, the desired deterrent effect may well be negated in favor of an antipodal one.

In further consideration of the deterrent effect of punishment on the potential murderer, we may contrast Illinois and Wisconsin during the period between 1931 and 1946. In the former, despite its use of capital punishment, four times as many murders were committed as in Wisconsin, which does not invoke capital punishment. The indifference of the criminal to the punishment ahead of him is the rule rather than the exception. In an attempt to illustrate the futility of capital punishment as a deterrent to homicide, Warden L. E. Lawes quotes one prisoner in the "death house" as saying, "The hot seat will never stop a guy from pullin' a trigger!"

Every profession has its own psychology and aggressions. The medical man aims his aggression at the affliction, while the lawyer seems to aim his aggression at the violator as well as the violation. Unfortunately, the criminal lawyer's training is deficient in that, unlike the doctor who works with his patients, he does not work with criminals, does not work in jails, or compare case histories. We must have an understanding of the varieties and vicissitudes of human aggressions or there can be no understanding of the criminal act.

The criminal act is an act of aggression, or more specifically, a hostility acted out. All human beings accumulate aggressive drives and we all must learn how to handle our unconscious aggressions. We must remember that a repressed aggressive drive does not necessarily disappear, it may merely become invisible to the outside world and unthinkable to the individual. These repressed aggressions and hostilities have a relatively free rein in the unconscious, cause diffuse anxieties and a consequential sense of guilt.

One way of handling these pent-up forces is to take refuge in fantasy. However, individuals occasionally give in to their fantasies and act them out, thus committing a crime. Aggressions turned inward result in suicide, while those directed outward result in homicide. Why one individual will give in to his fantasies and act them out while another will not, is a question that psychiatry finds unanswerable at the moment. The ways of human aggression are mysterious and often unpredictable and almost always undetectable except by careful, intensive work by a qualified psychiatrist.

The sensational case of Albert Fish is illustrative of this point. On the surface, to his neighbors, Fish was a pious Christian, while just barely beneath there lurked a personality that was literally savage and cannibalistic. A victim of mental disease, he was no longer a man, no longer a will. What do we accomplish by executing this sick man?

Presence or absence of capital punishment in a jurisdiction seems to have no effect on the homicide rate. Punishment is not a deterrent to criminal acts, hence our penal system is not so much a form of social defense as of hostility toward the criminal. The criminal trial is an adversary procedure and *lex talionis* reigns. The judge, rather than being truly impartial, because of his relationship with the state tends to identify with the prosecutor. A jury of twelve laymen is often called upon to "diagnose" the "sanity" or "insanity" of the defendant.

The law insists on differentiating "medical insanity" and "legal insanity," but we would never think of differentiating medical tuberculosis from legal tuberculosis.

Punishment alone cannot conceivably accomplish the avowed aims of the penal code. St. Thomas Aquinas said that punishment, to serve its purpose, must be acceptable to the one to whom it is meted out. In order for punishment to be acceptable as a rehabilitative mechanism, there must be a will, on the part of the one punished, in the direction of rehabilitation.

Penal codes exist and function at several levels. At the most primitive level, we find no sense of justice. At the next level, we find good deeds rewarded and transgressions punished. However, the punishment is usually out of proportion to the transgression. At the third and possibly ideal level, we find transgressions punished to teach and rehabilitate.

The public entertains ambivalent feelings toward medical men. The hostility to psychiatry in courts is quite marked due to objections to psychiatrists having difficulty reaching agreement on a patient's diagnosis. Psychiatrists, as men, are often in conflict through trying to correlate their medical knowledge and attitudes with legal requirements. This would be a difficult position for any human being. The fact that the court allows for a psychiatrist for the defense and one for the prosecution contains implicitly the idea that you can always hire an expert to bid for you. We also find that psychiatric "experts" are often "experts" by legal definitions only.

The psychiatrist should be retained as a friend of the court. No expert should appear for any one side. No expert should accept the concept of legal insanity. No psychiatrist should give opinions as to the responsibility or irresponsibility of a defendant since these are legal or moralistic questions. A panel of experts should be appointed by the court, from which three are to be chosen by the attorneys, who will retain the right to two pre-emptory challenges, as in choosing a jury. These should be empowered to deliver their reports to the court as individual or group opinions, with dissenting opinions being read as well.

J. M. Q.

CARDIOVASCULAR STUDY UNIT

January 13, 1954

AORTIC INSUFFICIENCY WITH SPECIAL REFERENCE TO ITS SURGICAL MANAGEMENT. By Charles A. Hufnagel, Department of Surgery, Georgetown University, Washington, D. C.

A mechanical method for correcting aortic insufficiency was described. This consists of inserting a valvular prosthetic into the descending aorta distal to the left subclavian artery. The device is made of a short section of methyl methacrylate tubing. A segment of this is enlarged to accommodate a movable polythylene ball, sensitive to small pressure changes because of its specific gravity of about 1.0. Systole pushes the ball down a distance equal to one-half its diameter and blood flows through channels around it. After systole, aortic back-flow returns the ball to the head of the valve and closes it. The position of the patient does not affect the function of the valve.

The prosthetic is fixed to the aorta by serrated nylon bands thus utilizing a multiple junction technique rather than the more traumatic equal pressure band. Necrosis with secondary hemorrhage has not been observed in any patient or experimental animal so treated. Insertion time is two and one-half to four and one-half minutes.

Forty patients with aortic insufficiency have been studied, all of them in serious preoperative condition. Most also had murmurs of mitral insufficiency. Some had mitral stenosis. All had cardiac enlargement and a life expectancy of not more than two years. Of these, one died operatively as soon as the chest was opened. Seven died postoperatively of various cardiac conditions. In the only case exhibiting postoperative embolus, thrombosis had occurred at the tubo-aortic junction and did not involve the moveable ball. The remainder returned to useful working lives. Their circulation time of T 1824 from right ventricle to femoral artery decreased to approximately normal limits. Fick cardiac output measurement increased but percentage changes varied greatly. On X-ray there was a definite decrease in heart size. Angina, which had been present in 50% of the patients was markedly reduced. Femoral diastolic pressure rose.

This is not offered as a conclusive answer to the problem of aortic insufficiency but as a preliminary report on a new concept of management of the graver cases.

P. B., JR.

BEAUMONT CLUB

November 20, 1953

RAMÓN Y CAJAL AND SHERRINGTON. By John F. Fulton. Department of the History of Medicine, Yale University School of Medicine.

Cajal and Sherrington met in 1885 when both were studying an Asiatic cholera epidemic in Spain. Their relationship was characterized by mutual esteem and great reciprocal influence, scientifically. Parallelisms in their lives include early papers on inflammation, on neuroanatomy, and a lifetime spent in investigation of the nervous system, Sherrington concentrating on function, Cajal on structure. Inspired by Simarro's Golgi chrome-silver preparation, Cajal went on to develop the neurone doctrine and became universally renowned. His early papers are now difficult to find outside of Spain. Invited to address the Royal Society in 1894, Cajal was

Sherrington's guest while in London. Sherrington wrote the official obituary after the great Spaniard's death in 1934.

M. C.

NEUROLOGICAL STUDY UNIT

January 12, 1954

SOME NEUROPSYCHIATRIC ASPECTS OF THE STUDY OF LANGUAGE. By Stanley Cobb, Professor of Neuropathology, Harvard Medical School, Boston, Massachusetts.

Subhuman "language" most closely approaches our own when it expresses emotion. In its other forms it represents an innate pattern of response, occasionally giving a specific message, but never leading to any exchange.

Human speech on the other hand does not require a previous experience, but can well take its place. It is basically an exchange, and has an implied as well as a manifest meaning.

Hoping that the manner of talking will give information about the personality of the speaker, word frequency, speaking rate, phrase length, and spontaneity were studied. Lobotomies and gyrectomies resulted in a shortening of phrases, slowing of speech, and reduction of spontaneity. Neurotics and manics used few new words, while the use of pronouns reflected external or internal orientation of the patients. Analysis of language based on such previously established standards correlated well with Rorschach interpretations.

N. A. H.

ZOOLOGICAL JOURNAL CLUB

December 15, 1953

PITUITARY, ACTH, AND CORTISONE IN WOUND HEALING AND REGENERATION IN THE AMPHIBIA. By Oscar Schottë, Amherst College, Amherst, Massachusetts.

In the 18th century, with the cell theory as yet undiscovered and morphology in an unsettled state, biologists were unprepared to investigate the phenomenon of regeneration. It became regarded as a specific property, and organisms were classified in terms of those capable of regeneration and those incapable of it. We shall show that the organism's ability to regenerate is dependent on certain systemic and local conditions, and does not constitute a particular property.

Amphibian larvae can regenerate amputated limbs in the absence of the pituitary, but hypophysectomy in an adult prevents regeneration. Cellular dedifferentiation and proliferation may occur, but this results only in the formation of a cellular "cushion" at the site of amputation beneath the epidermis which seems to block any regenerative growth. If the limb is permitted to begin regeneration prior to hypophysectomy, a minimum amount of regeneration may occur, the degree varying with the interval

between hypophysectomy and amputation. A ten-day interval will allow a superficially normal limb to develop. We conclude that the pituitary is necessary to initiate regeneration in the adult, but that the blastema's growth can *proceed* in its absence.

Injection of cortisone, desoxycorticosterone, or ACTH permitted regeneration in the pituitary's absence, although ACTH injected into a normal control retarded growth.

Local conditions of stress, e.g. the application of salt to the amputation site, produced different effects in the presence of the same factors.

Amputation of the limb of a normal animal will lead to regeneration if the local conditions are favorable. In the absence of the pituitary, regeneration may still occur if replacement therapy is instituted (adrenalcortical extracts). In the absence of either the pituitary or replacement therapy, there is no regeneration, though special local chemical or physical conditions may modify this. Regeneration and nonregeneration answer to a whole series of conditions, both local and systemic. The regeneration of the larvae in the pituitary's absence is to be explained in terms of these organisms' unpreparedness to alter their systemic conditions (e.g. they evince no stress reaction). Regeneration is here determined by local factors alone.

P. K.

January 6, 1954

ETIOLOGIC MECHANISM OF A FORM OF GENETIC OBESITY. By Jean Mayer, Harvard School of Public Health, Boston, Massachusetts.

According to the glucostatic theory, there are two lateral centers in the hypothalamus which respond to blood sugar levels and lead to sensations of hunger when the arterial-venous levels differ by more than 10 mg./100 cc. Thus, since blood sugar levels are dependent upon the complex process of rate of glucose utilization, various biochemical centers are involved in the problem of hunger and food intake. Neural factors are involved, too, for the hypothalamic impulses must be integrated at the cortical level. With so complex a control, numerous factors—genetic, traumatic, or environmental —can alter the balance and lead to obesity or emaciation.

In the mouse, a Mendelian recessive condition of genetic obesity has been studied which differs considerably from obesity promoted by chemical or operative means. Gross behavioral changes are evident which are not due simply to the accumulation of fat: the animals show no sex interest (reproduction must be via artificial insemination), and there is little spontaneous activity. When allowed to select their own foods, they chose a diet whose composition corresponds to that of mouse-milk. (These obese individuals, therefore, would seem to meet the criteria for fixation at the oral level, as proposed by some psychiatrists.)

The amount of food eaten by obese individuals is not great enough to account for their increased weight. This increase is due largely to reduced oxidative rates and exercise. In connection with this, it should be said that the obese mice cannot tolerate cold and die rapidly when exposed to it.

Shivering and pilo-erection occur, but there is no increased oxidation. They are hyperglycemic or else can have hyperglycemia easily induced by injections of growth hormone to which they are highly sensitive. They are resistant to insulin. How is it that so complex a syndrome is produced by a single gene?

Destruction of the alpha cells of the Isles of Langerhans led to a reduction of obesity and reversion to normal behavior. It was concluded, therefore, that the obesity syndrome was probably due to a pancreatic hyperglycemic factor secreted by the alpha cells as well as a concomitant increase in beta cell (insulin secreting) activity. In the obese mouse, the two secretions are in equilibrium, but their high level produces the obese syndrome.

P. K.

January 27, 1954

EXPERIMENTAL ANALYSIS AND ALTERATION OF COCOON SPINNING BEHAVIOUR IN SILKWORMS. By William G. Van der Kloot, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

Harvard's Law of Animal Behaviour ("Under precisely controlled experimental conditions, animals do what they damn please") is belied by the stereotyped spinning patterns of silkworms. The larvae of *Cecropia,* the silkworm, hatch in the spring, moult thrice, attaching their cuticle to a twig by means of a silk pad the third time, and finally pupate, to remain in diapause until the following spring. The spun cocoon consists of a mile-long thread of protein produced by a pair of much-convoluted silk glands and has a specific structure; two layers, a cone shape, and an escape valve at the upper end. The cocoon-building behaviour is initiated by the secretion of prothoracic hormone in the absence of the secretion from the corpora allata. In the presence of both substances, the silk pad of the third moult is produced, while the removal of the corpora allata from an immature animal will result in the precocious production of a normal cocoon. The spinning behaviour, in other words, is potentially always present.

The movements in the manufacture of the cocoon were shown to be rigidly stereotyped, though well adapted to a three dimensional environment. If the animals are placed in a two dimensional world (e.g., the inside of a balloon), they may spin a flat sheet of silk, though the inner envelope of a cocoon will be spun upon their removal. That is, the distribution of the silk into two layers is part of the normal pattern, and this is a pattern that can be distorted only by altering the environment or interfering with the associated enteroceptive stimuli (e.g., removal of the silk glands).

The behaviour patterns are species specific and are probably due to genetically determined central nervous mechanisms. Micro-surgery has indicated that the centers for the spinning behaviour are associated with the roots and cell bodies of the corpora pedunculata. More precise localization may be possible in the future and may provide more knowledge of how patterned nervous activity is translated into behaviour.

P. K.

February 3, 1954

THE BREEDING BEHAVIOUR OF SOME INDIAN WEAVER BIRDS. By Sálim Ali, Secretary of the Bombay Natural History Society, India.

Indian weaver finches (family, *Ploceidae*) construct suspended tube-shaped nests, usually amidst the branches of palms or accecias. As many as 250 nests may be found in a single tree, generally overhanging a body of water, while identical trees nearby may be quite unused. This same tree is repeatedly chosen in succeeding years.

The nest material consists of rachet-edged strands from the immature rice plant, specific plants being selected by a party of nestbuilding males as their source. The migrant females arrive after the nests have been partially completed, and, after a deliberate process of inspection, choose one, which is then rapidly completed by the male. Relations are polygynous, with a male having as many as three to four nests, and, subsequently, families.

Each mated female produces two to four eggs, which are incubated for a 12-day period. During fledging, the males take little part in the care or feeding of the young. At night, in fact, the males roost in special sites away from their nests where they are joined by the nonbreeding females.

Damage to the nests by crows, rats, or pocket-knife-armed ornithologists is quickly repaired. Generally, these intruders cause no significant mortality. Man, as a consequence of the finches' rice-eating habits, is their greatest enemy.

Studies were made from raised observation tents and were augmented by color motion picture films.

P. K.

ZOOLOGICAL AND BOTANICAL JOURNAL CLUBS

January 13, 1954

EXPLOSIVE EVOLUTION. By Paul C. Mangelsdorf, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

It is usually true, as ordinarily thought, that evolution proceeds at a leisurely pace. The rate of evolution of domestic species, however, may proceed much more rapidly. Darwin recognized this, too, and thought that this represented a magnification of the usual process.

There are many examples of the rapidity of evolution under domestication, the extremes of size and shape among dogs as demonstrated by whippets and dachshunds *vs.* St. Bernards and Yorkshire terriors being but one. The factors affecting this evolution are the following: mutations, which seem to occur more frequently among domestic species; macromutations, which, in nature, would not survive; random genetic drift; artificial selection; and hybridization. A study of a particular case, namely that of maize, is illustrative of the changes that can occur.

Modern maize probably would not survive for more than one year if not cultivated, yet it arose from hardy primitive types. Studies of carbonized particles of corn, including some nearly whole cobs, found in various caves by archeologists, permit us to reconstruct early maize. Apparently it was a

short stemmed, loosely packed plant, with long floral grass and small kernels (about the size of our smallest popcorn). This form may have arisen from a fertile homozygous pod-corn which bore no ears. The next step in evolution probably was the formation of cob-like pods, an enlargement of the rachis and reduction of the glumes which provided for a more efficient nutrient supply mechanism as well as for an increase in the number of rows of kernels and their size. The production of modern varieties came as the result of macromutations on chromosome four.

It is apparent that random genetic drift has played a rôle in maize's evolution as is seen from the definite distributive patterns of the Pr and I genes, neither of which have any adaptive value. Hybridization has also been of considerable importance, the crossing of the early type with Teocinte corn giving the rachis the requisite hardness. In addition, this hybridization with Teocinte appears to have had a mutagenic effect.

The difference between evolution of wild forms and the changes taking place under domestication are differences in rates only. The mechanisms involved are essentially identical.

P. K.

PLANT SCIENCE SEMINAR

January 25, 1954

SOME INTERESTING NUCLEAR ASSOCIATIONS IN THE FUNGI. By Lindsay S. Olive, Department of Botany, Columbia University, New York, New York.

The terms "homothallism" and "heterothallism" were first used by Blakesley to describe the types of sexual reproduction found in certain of the Mucorales. It is generally accepted that a homothallic fungus is one which is able to complete its life cycle when grown from a single haploid spore, whereas an heterothallic fungus requires the pairing of two complementary strains in order to reproduce sexually. Whitehouse, in a recent review, has somewhat modified Blakesley's terms so that the heterothallic condition has now been subdivided into "morphological heterothallism" and "physiological heterothallism."

However, several recent investigations have shown that in some instances fungi cannot be considered exclusively homothallic or heterothallic. The work of Olive, *et al.* with *Glomerella cingulata* illustrates that what is normally considered a homothallic species may give rise to behavior normally associated with heterothallism. Mutants with which they worked are listed in the table.

Glomerella cingulata

Clumped perithecial	(1) unstable	$A^+ B^+ M$
	(2) stable	$A^+ B^+ m$
Scattered perithecial	(3) fertile	$A^+ B^1$
	(4) infertile	$A^+ B^2$
Scattered conidial	$A^1 B^1$ (do not form perithecia)	
Clumped conidial	$A^1 B$ (do not form perithecia)	

[Where M causes mutation, A^1 no perithecia and B is the locus for "clumping."]

When A^+ B^+ strains were crossed with conidial strains, genetic analysis showed that the presence of wild-type had induced perithecial formation in the conidial strains since asci gave rise to all types including conidial strains. This happened even when a suppressor gene was introduced into the wild-type before crossing with the conidial strains. Similar behavior is shown by the work of the Pontecorvo group with *Aspergillus,* of Dirk and Raper, and of Hirsch with *Hypomyces.* Hirsch's explanation appears untenable in this instance, since in all of the asci that were investigated the number of chromosomal pairs was constant. Microscopic observations of sexual reproduction in *Glomerella* were illustrated by a series of slides of photographs and camera lucida drawings.

In conclusion: (1) sexual processes in fungi are flexible, (2) some species cannot be classified exclusively homothallic or heterothallic, (3) heterothallism has given rise to homothallism and vice versa at various times in evolution, (4) heterocaryogamy and segregation are common in homothallic fungi and are of survival value.

D. BOULTER

PLANT SCIENCE-ZOOLOGY SEMINAR

January 11, 1954

POLYTENE CHROMOSOMES AND DIFFERENTIATION. By Professor Crodowaldo Pavan, University of São Paulo, Brazil.

The general problem is the manner in which cells, originally of the same genotype, differentiate in the development of the organs of multicellular animals. There is evidence that this development is in part determined by the cytoplasm and in part by the nucleus.

Three types of nuclear differences have been observed during differentiation—elimination of all or parts of chromosomes, as in *Ascaris* and certain diptera; replication of parts of chromosomes, as in many diptera; and differences in behavior of nuclear material, as in the nuclei of *Paramecium.*

One can also look for differences in the band patterns of polytene chromosomes from different tissues of dipteran larvae, and for banding differences in the same tissues at different stages of development. It has been found that the dipteran *Rhynochosciara angelae* is ideal material; the larval salivary glands are as long as the larva itself, which is two cm., and the chromosomes are not only correspondingly large but well defined throughout development. Polytene chromosomes are also found in the intestine, the Malpighian tubules, and certain muscles. It is also possible to follow the chromosomes throughout the development of the larvae because the 100 to 500 eggs are laid in a period of about 40 minutes, the larvae are gregarious and pupate together and hatch some weeks later in a period of three hours.

The principal observation (illustrated by photographs and slide projections) is that chromosome bands may become diffuse and expanded, giving a "puffy" appearance to that region of the chromosome. They then return

to the condensed condition with the appearance of bands. Heterochromatin is accumulated just before the "puffing" begins. Different regions of the chromosomes display this behavior in well-defined periods of larval development and pupation. The "puffing" is not synchronous in different tissues.

The interpretation is that the same genes are working in all tissues of the animal, but that their activity is neither synchronous nor exactly the same in each place, but is in part determined by the cytoplasm. The evidence is believed to support the hypothesis that euchromatin and heterochromatin are not different substances, but different states of activity of the same substance.

R. R. L. G.

YALE MEDICAL SOCIETY

January 21, 1954

ATTENUATION OF POLIOMYELITIS VIRUS ON PASSAGE THROUGH TISSUE CULTURE. By Joseph L. Melnick and C. Alan Philips, Section of Preventive Medicine, Yale University School of Medicine.

Serial passage of poliomyelitis virus through cultures of non-nervous tissue should favor the selection of non-neurotropic variants which may arise. Enders has already shown that the Brunhilde strain (Type I) could be modified by passage through human tissue cultures so that its virulence for monkeys was greatly decreased. It appears that mutations, to produce variants with decreased neurotropism, constantly occur in tissue culture with strains of all three antigenic types. Representative strains of the three antigenic types of poliomyelitis virus have been studied. Both the ability of the three types to produce experimental poliomyelitis in monkeys intracerebrally inoculated and their ability to propagate in cultures of monkey testicular and kidney tissues have been used as markers. Strains with decreased virulence for monkeys have been obtained by making a number of serial passages at high concentrations of virus followed by passage at limiting dilutions. Intramuscular inoculation of monkeys with such attenuated viruses have not produced disease, although the monkeys were left with antibodies capable of neutralizing antigenically related virulent strains.

A partially attenuated Type I strain was compared with the original neurotropic strain from which it was derived for its ability to infect chimpanzees by the oral route. It was found that a virus which is partially attenuated may no longer be infectious by the oral route, or, if infectious, may revert in the course of its multiplication in the chimpanzee to its original virulent state.

JOSEPH L. MELNICK

THE FINE STRUCTURE OF NEURONS. By Sanford L. Palay, Department of Anatomy, Yale University School of Medicine.

Neurons from sympathetic and dorsal root ganglia, medulla oblongata, and cerebral cortex of living anesthetized rats were examined in thin sections. Tissues were fixed in 1-3% buffered osmium tetroxide and imbedded

in n-butyl methacrylate. Electron micrographs showed compact masses of Nissl substance to be the most conspicuous cytoplasmic features. The two components of Nissl bodies, as of cytoplasmic basophilic material in other cells, are: (1) a thin, membranous endoplasmic reticulum arranged as more or less parallel anastomosing tubules or flattened vesicles, and (2) punctate granules which lie upon or between the tubules or vesicles. The cytoplasm between Nissl bodies contains rounded lipid inclusions, numerous mitochondria, and fine filaments 6-10 mμ in diameter and of indefinite length. A second system of membranes is distinguished from that of Nissl bodies by narrower lumina, tighter packing among membranes and absence of granules. There are intermediate forms between the two membrane systems. Dendrites and axons have similar structures, except for the extension of the Nissl substance into dendrites for a short distance. The endoplasmic reticulum loses its associated granules and tapers into parallel slender strands. Occasional mitochondria and numerous fine filaments like those in the perikaryon are found among these strands. A collection of mitochondria and small vesicles (20 mμ in diameter) fill the axon termination. The cell membrane at this site is thicker and denser than elsewhere.

G. A. F.

ORBITAL UNDERCUTTING IN THE TREATMENT OF PSYCHONEUROSES, DEPRESSIONS AND SENILE STATES. Results of fractional lobotomy in the milder emotional illnesses. By William B. Scoville, Hartford, Connecticut and V. Gerald Ryan, Portland, Connecticut.

Five years ago, the writer presented a new method of fractional lobotomy permitting precise isolation under direct vision of various areas of frontal lobe cortex by means of cortical undercutting. The results, chiefly in schizophrenic patients, were summarized in 1951. Undercutting of the inferior or orbital surface of the frontal lobes has been carried out in 112 cases and it was noted that when undercutting was limited to this area, no appreciable personality blunting or deficit occurred. This has recently been confirmed by the studies of Rylander and Sjöqvist. For this reason, selective cortical undercutting of the orbital surface of the frontal lobes has been done in 34 cases of the milder emotional and mental illnesses, including obsessive-compulsive neuroses, anxiety-tension states, somatic conversions, cyclic depressions, and senile emotional disturbances. The results have been gratifying in all categories, resulting in a lessening of anxiety and tension, a fading of morbid obsessive thinking, a lightening of mood, frequent strengthening of male libido, and relief from drug and alcohol addiction provided they are secondary to anxiety or panic states. Fractional lobotomy should not be performed on constitutional alcoholic, psychopathic, or criminal types.

To our surprise, the senile emotional disturbances have shown the greatest benefit. In brief, it appears that the older the neurotic or depressed patient the more he will benefit by any form of lobotomy sufficiently limited to prevent personality deficit. In those agitated, sleepless, and involutional cases thought to be incapacitated by the destructive processes of old age, deliberate destruction of an additional portion of the frontal lobe has re-

stored them to a happy and frequently useful existence. This is a cause for surprise and surmise as to the deeper dynamics of senility. Twelve cases over 65, three being over 80 years of age, have been subjected to orbital undercutting with significant benefit and without appreciable deterioration.

The degree of improvement in all categories has been in the following diminishing order: (a) senile emotional states, (b) depressions and affective illnesses, (c) psychalgias, and (d) physical conversion states including headache, tinnitus, vague pain, and ulcerative colitis. Organic pain is insufficiently benefited. There have been no adverse effects in the release of hostility or loss of social sensitivity except for an uncovering of an underlying schizophrenia, which occurred in seven out of 34 cases. These have been aptly labeled pseudoneurotic schizophrenia by Kalinowski. Eventual benefit occurred to both the neurotic overlay and the psychosis. There have been no infections, one death from coronary thrombosis; 5% had isolated convulsions, permanently arrested by dilantin. In contradistinction to the standard lobotomy, improvement is insidious without any abrupt change in the over-all personality.

W. B. S.

THE Fe^{59} TURNOVER RATE OF CYTOCHROME C. By S. C. Finch and J. F. Ross, Department of Internal Medicine, Yale University School of Medicine.

The cytochrome *c* turnover rate in the livers, kidneys, and hearts of rats was determined, using radio-iron techniques. Following the intraperitoneal injection of Fe^{59} to a group of albino rats, animals were sacrificed at weekly intervals, cytochrome *c* was isolated, and the radio-iron content determined.

The studies indicated that the turnover rate of this heme enzyme in these tissues was much more rapid than the rates reported by others for guinea pig striated muscle. The studies also showed that cytochrome *c* turnover in the heart greatly exceeded that of either the liver or kidney. The half-time disappearance of Fe^{59} from heart cytochrome was 1.8 days, while that of liver was 5.8 days and kidney 8.0 days.

Contrary to previous iron metabolic concepts, these results suggest that heme pigment iron may interchange freely with body iron, and that the rate of cytochrome *c* production and utilization may be related to tissue respiration.

S. C. FINCH

February 8, 1954

PROBLEMS IN WOUND HEALING. By Edward D. Churchill, John Homans Professor of Surgery, Harvard Medical School, Boston, Massachusetts.

The effective surgical therapy of wounds depends on an understanding of the natural processes of healing occurring without external interference. The initial enzymatic and phagocytic process of removing nonviable tissues can be eliminated in non-contaminated wounds by débridement. However, as war experience has shown, repair will not start even in a clean wound for four or five days, and advantage can be taken of this fact by the method of delayed suture after this time has elapsed. It has been shown experimentally that in this period there is no increase in the tensile strength of

the wound, nor is there any reduction of its area by shrinkage or epidermization.

Healing can take place by two processes: scarring or regeneration. Usually cicatrization, or what might be better termed shrinkage, is the first to occur. It may be followed by organization and regrowth of the original structures on the scaffolding of scar tissue. The latter is influenced by the conditions existing in the wound, such as stresses and tensions. Future developments in surgery may give us mastery of directing healing processes, permitting repair or regeneration of structures hitherto considered irreplaceable.

N. A. H.

ANATOMY SEMINAR

February 16, 1954

MIGRATION OF LARGE MAMMALS. By Dillon Ripley, Peabody Museum, New Haven, Connecticut.

Territoriality among animals was first discussed by Aristotle with reference to the hunting territory of the hawk. Thereafter, the concept received little further attention before the seventeenth and eighteenth centuries. Ordinarily, it applies to any defended area which is usually a specific biotope within the animal's geographic range.

Hippopotami travel in herds led by a dominant male. Fragmentation of the herd occurs at the breeding season with each male defending a separate territory together with a harem of several females. This territory, from four to five square kilometers in area, is demarcated by urine trails and by the feces, which is fragmented and spread by the tail as it emerges.

Elephants remain in herds throughout the year, one male dominating the herd and generally serving all the females. Rogues, outcast males, exist apart from the herd, either singly or in pairs. Both types of groups occupy specific feeding territories.

Rhinoceroses remain on individual territories throughout most of the year. These are demarcated by dung heaps as much as four feet in height and urine trails, the latter being produced by the animal's posteriorly directed penis, as is the case with hippos. At the advent of the mating season the territory is abandoned and a mate sought. This is done by both sexes. During this time the rhinos may wander hundreds of miles. This occurs more generally in the spring, but since the latitude of their home ranges is about 25° N. (India), there are no external synchronization mechanisms, and mating may occur at any time. Thereafter, the animals return to the vicinity of their original territory.

Among these larger mammals there is generally no social coöperation though coöperation plays an important rôle among other mammals. Presumably this is due to coöperation having no survival value for animals with no prey-predator relations. Hippos, rhinos, and elephants have no enemies, though they get along poorly with one another (moral: if you hunt rhinos on elephant-back, pick one with tusks). Play, which is not exhibited by these mammals, is of importance in building social relationships, too.

P. K.

BOOK REVIEWS

HUNTED HERETIC; THE LIFE AND DEATH OF MICHAEL SERVETUS 1511-1553. By Roland H. Bainton. Boston, Beacon Press, 1953. xii, 270 pp. $3.75.

The Reverend Roland H. Bainton is the distinguished Titus Street Professor of Ecclesiastical History at Yale University. For over twenty-five years Mr. Bainton has been studying about and publishing books on the four principal heretics of the Reformation who, as he puts it, "fled from Catholic countries to the Swiss cities and there fell foul of the Calvinist regime." The *Hunted Heretic* is the last of four monographs on the heretics; Mr. Bainton's earlier monographs on Castellio, Joris, and Ochino appeared respectively in 1935, 1937, and 1940. This present work appeared on the 400th anniversary of the burning of Servetus at the stake in Geneva on 27 October 1553.

Servetus was one of those men of the sixteenth century who was active in many fields of knowledge. He was linguist, Biblical scholar, theologian, geographer, physician, and physiologist. It is as physiologist that he is of most interest to biologists and physicians today; Servetus independently discovered the pulmonary transit.

Mr. Bainton has written a very readable book. He has made extensive use of original sources both printed and manuscript, and he has achieved a remarkably scholarly and interesting interpretation of Servetus. I use the phrase "remarkably scholarly" because even a casual acquaintance with Servetus literature will convince anyone that perhaps as much fiction has been written about him in the guise of fact as about any other one man. *Hunted Heretic* is an outstanding exception.

The history of the discovery of the pulmonary transit is also replete with conjecture. In fact the whole story seems to have gotten off on the wrong foot (right or left?) when William Wotton first publicized Servetus' discovery in his *Reflections on Ancient and Modern Learning* (London, 1694). In this work Wotton reported "he [Servetus] clearly asserts, that the Blood passes through the Lungs, from the Left to the Right [!] Ventricle of the Heart." Mr. Bainton's discussion of Servetus' discovery, like the rest of his book, is interesting and accurate. It is the best brief statement available.

FREDERICK G. KILGOUR

THE BOOK OF HEALTH. Compiled and Edited by Randolph Lee Clark, Jr. and Russell W. Cumley. Houston and New York, Elsevier Press, 1953. 843 pp., 1400 illustrations and plates. $10.00.

"Medicine is no longer a secret science. Every newspaper and magazine publishes information and stories about progress in medicine. Sometimes, this information is premature or unreliable; often it raises false hopes. . . ." "This book tells what 242 doctors [many of them eminent] believe you and your family should know about medicine. . . . what your physician wants you to know about your body—how to keep it healthy and what to do in case of illness." These quotations from the jacket indicate the purpose of this book.

Often the reviewer has wished for such a book to recommend to an intelligent lumber boss responsible for the health of his men in camp deep in the north woods, sometimes isolated by blizzard; a non-medical missionary taking his wife to a post in Central Africa three days by dugout canoe from the nearest physician; an anthropologist going to study some primitive tribe in the hinterland; a capable grandmother, living forty miles from the nearest physician and sixty miles from the nearest hospital, who, picking up medical lore from radio and weekly papers, is the first to be called on when illness strikes in the little community; and a part-time household helper who wishes she had a book to check up on "what they say on the radio."

The Book of Health answers the need with scientific integrity except, perhaps, for too great enthusiasm for some of the most recent and still controversial procedures which may soon be out of date. The excellent diagrams and illustrations depicting the text should prove helpful.

Unfortunately the huge bulk, due to the many historical illustrations and color plates, make the book unduly expensive and unmanageable while adding little to its real usefulness.

ARTHUR BLISS DAYTON

CHYMIA: ANNUAL STUDIES IN THE HISTORY OF CHEMISTRY. Vol. 4. Henry M. Leicester, Editor-in-Chief. Philadelphia, University of Pennsylvania Press, 1953. viii + 217 pp.

In 1948 the first volume of *Chymia* was published by the University of Pennsylvania Press. At that time it was announced that *Chymia* would be a yearly publication intended "to promote international scholarship in the history of chemistry . . . and to provide a meeting ground for those who find pleasure in studies such as it reports."

Since that time four volumes of *Chymia* have appeared. Of these volumes, the fourth is undoubtedly the worst. Although every volume was somewhat irregular in the quality of the papers contained in it, there were always one or two papers which served to justify the entire venture. Unfortunately, this saving feature is noticeably lacking in volume 4. Beginning with a rather pedestrian and uninspiring paper by Professor R. J. Forbes "On the Origin of Alchemy" and ending with a semi-sociological survey of "The Scientific Background of Chemistry Teachers in Representative Pharmacy Schools of the United States During the 19th Century" by Professor Glenn Sonnedecker, this volume of essays is almost uniformly mediocre and, what is far worse, dull. In some cases the papers are simply bad and do not deserve the immortality which the excellent paper and binding of this volume will guarantee them. In others, the papers are relatively competent but uninspired.

The essays in this collection may be divided roughly into two categories; those which make no claim to any great originality or scholarly value, and those which are the obvious products of extensive research and which, therefore, must be judged according to the standards of modern historical scholarship. In the first category fall Professor Forbes' article on alchemy, Denis Duveen's chatty and thoroughly innocuous paper on Madame

Lavoisier, Curt Bühler's bibliographical article "A Projected but Unpublished Edition of the 'Life and Works' of Robert Boyle," and Professor Ganzenmüller's article on the writing of the history of alchemy. These articles are relatively pleasant, even, as in Professor Ganzenmüller's case, stimulating, but one feels curiously empty after finishing them. They might serve as introductory essays to various areas of the history of chemistry, but seem out of place in what is obviously intended to be a serious yearly publication on the advanced and difficult aspects of the history of chemistry.

The second category of papers presents a more serious problem and requires a more detailed analysis. Almost one-third of the entire 200-page volume is occupied by two articles which will serve as examples of the general shortcomings of the "scholarly" articles. The first article—Wyndham Miles' "Benjamin Rush, Chemist"—is just simply a bad paper. It occupies forty pages and is in desperate need of editing. Professor Miles has tried to describe Dr. Rush's chemical activities in these pages but in so doing has become so inextricably entangled with the history of the medical faculty of the College of Philadelphia and the question of the place of chemistry in late eighteenth century American medical education that he never manages to deal with any one of these problems adequately. The result is a highly confused and poorly constructed essay almost totally lacking in literary value and, because of its confusion of purposes, of little use to the historian of chemistry.

Professor Glenn Sonnedecker's aforementioned paper on the scientific background of chemistry teachers represents the opposite extreme from Professor Miles' effort. Where Professor Miles is guilty of casting his scholarly net too widely, Professor Sonnedecker has pursued a single idea with monomaniacal intensity through thirty heavily documented pages and twice as many eventful years in American history. There is no doubt that Professor Sonnedecker's paper will be of use to anyone interested in the history of American pharmaceutical education but here again the services of a good editor would have been welcome. The paper could have been cut with great gain; repetition of facts and long lists of names are not necessary and certainly will not arouse the interest of the average reader. Finally, the use of pseudo-sociological method and terminology leads, in this reviewer's opinion, to poor sociology and even poorer history. Again, as with Professor Mile's article, the raw material for a good paper is present; it is the author's job, however, not the reader's, to transform this raw material into a finished product.

These criticisms of Volume 4 of *Chymia* should point out what seems to this reviewer to be a basic problem which the editors must face. They must decide for whom these articles are intended. Are these essays addressed to the professional historian of science, to the chemist interested in the history of his science, to the general historian conscious of the effects of the rise of chemistry on other branches of history, or to the intelligent layman? The type of article accepted by the editors of *Chymia* will obviously be affected by their decision on this question and their choice will, it is to be hoped, give this publication a unity which it now lacks. But regardless of what type

of audience is assumed for these volumes, the editors have the responsibility of maintaining a certain minimum standard of literary style and must take their editorial duties more seriously by refusing to publish papers which do not come up to this standard.

L. PEARCE WILLIAMS

RENAL FUNCTION. Transactions of the Fourth Conference sponsored by the Josiah Macy, Jr. Foundation, October 22-24, 1952. Stanley E. Bradley, Ed. New York, 1953. 189 pp. $3.50.

This is presumably a play-by-play report of a scientific conference staged according to a peculiarly American pattern widely featured by the radio in its educational and informational programs. The sponsor, duly acknowledged by a spokesman, is in this case a philanthropic foundation. The symposium actually deals chiefly with the nature of ionic transfers across membranes in various organs of the body, rather than renal function per se. The text is divided into four parts. The first, Ion Exchanges Between Extracellular and Intracellular Fluids, contains relatively formal presentations by Robert F. Pitts, Roy C. Swan, William Wallace, and Daniel C. Darrow; the other three are devoted to more formal talks by Gilbert H. Mudge on Cation Exchanges in the Renal Equilibrium, Hans H. Ussing on Ion Transport Across Living Membranes, and Maurice B. Visscher on Water and Ion Movements Across Intestinal and Renal Epithelium. A distinguished company participates in the discussion of the papers.

According to the opening remarks of the Medical Director, Frank Fremont-Smith, the ostensible purposes of the conference are "furthering knowledge in a particular field"—i.e., renal function—and "promotion of meaningful communication between scientific disciplines." One wonders whether in this era, when means of communication have been so greatly improved, multiplication of such conferences promotes the advance of science so much as it diverts the scientists from productive activities. Besides the three formal speakers named in the Table of Contents, a number of the selected audience are permitted or invited to participate in the discussion. More than the usual number of these seem to have happened upon slides in their pockets. In fact, one of the most provocative contributions in the series, by Pitts, appears in the first chapter, which is accredited to "Informal Discussion." This serves as the introductory scientific address. The major part of the material presented in the volume is not new; a large proportion of the figures and tables are taken from published articles. These are sometimes supplemented by the speakers or the adventitious illustrations produced by the participating guests. Some novelty is lent to the presentations by the discussion, which is for the most part presented in an informal manner. Much of this is tantalizing and provocative, rather than satisfying, because few of the participants are permitted to develop their ideas. Sometimes these get no further than being presented and "kicked around." That they are treated in reasonably good English style must probably be credited to the skillful offices of the editor. Though this adds to its elegance, it is presumably one of the reasons that the volume appears one year after the

conference. A simple transcription from a wire recording might embarrass the participants and violate aesthetic sensibilities of the readers, but it would expedite the publication of matter that is inherently dated.

These are all natural weaknesses of this particular vehicle, which is the present vogue. Among published reports of conferences, the one in question ranks high, maintaining the standards set by the Macy Foundation Conference reports. The subject is opportune; the chief speakers and the participants are able; most of the presentations are concise and lucid. Their brevity, however, compels them to be starkly factual. The discussion, since it is impromptu and casual, is dispersed and fragmentary. An occasional penetrating, philosophical remark is left tantalizingly suspended by diversion. On the whole, despite the ability of the participants, the discussion is dilute. No doubt these remarks provoked profitable exchanges in the cloak room or at the bar to the advantage of some of the individuals present; but these are lost to the reader. This well-edited report of an efficiently organized and conducted conference on an important subject illustrates certain well-established educational principles. It is possible to read in ten minutes what can be spoken in an hour and, while reading, stop to reflect on the subject matter at will. The number who can profitably engage in conversational discussion of any important subject is strictly limited.

JOHN P. PETERS

SOME ASPECTS OF ENZYME RESEARCH. F. Dickens, Ed. London, British Medical Bulletin, Volume 9, Number 2 (1953). $2.75.

During the past nine years, *The British Medical Bulletin* has appeared in the form of a series of symposia on topics of current interest in medical research and practice. The issue under review is one of the best in the series, and contains fifteen excellent articles. The first of these, on "Cytochrome," by D. Keilin and E. C. Slater, is especially recommended as a well-balanced and authoritative account of a field in which brilliant contributions have often been marred by polemic. This article is followed by one by H. A. Krebs on "Some aspects of energy transformation in living matter," a stimulating integration of current knowledge and thought on this important subject. The succeeding articles are by F. Dickens on "Alternative routes of carbohydrate oxidation"; by M. Dixon and E. C. Webb on "Phosphate-transferring enzymes"; by Sir Rudolph Peters on "Significance of biochemical lesions in the pyruvate oxidase system"; by D. D. Woods on "Folic acid and related compounds in the metabolism of micro-organisms"; by G. A. Levvy on "β-Glucuronidase and related enzymes"; by Charles S. Hanes on "The formation of peptides in enzymic reactions"; by E. F. Gale on "Amino-acid decarboxylases"; by R. H. S. Thompson on "Cholinesterases"; by J. H. Quastel on "Action of drugs on enzyme systems"; by H. Blaschko on "Enzymic oxidation of amines"; by Sir Cyril Hinshelwood on "Adaptive enzymes"; by J. N. Davidson on "Enzymes acting on nucleic acids"; by E. J. King on "Plasma alkaline phosphatase in disease."

Although all the articles are written by men in the British Commonwealth and British work consequently receives emphasis, in every case due account

is taken of other important work that bears on the subject. The issue thus provides a valuable summary of some recent advances in biochemistry and is highly recommended.

JOSEPH S. FRUTON

THE SPINAL CORD. Ciba Foundation Symposium. Boston, Little, Brown and Co., 1953. xii + 564 pp. $7.50.

The organizers of this conference got off to a good start by inviting investigators, both old and new, who have been engaged in the study of various aspects of cord neurophysiology, wisely restricting the field to the regions up to the medulla oblongata.

The conference was almost unique in inviting papers on the morphology and histology of the cord, stressing the crucially important problem of motor neuron growth and organization, especially the rôle of peripheral connections on the general morphogenetic behavior of neurons in the cord. The emphasis of the relationship of structure to function was set by the early papers and apparently was sustained throughout the session both in the ensuing papers and the discussion periods. The autstanding example of the relationship of histological structure of neuron pools and electrophysiological response is present in Lorente de Nó's masterful paper dealing with the electrical activity of the oculomotor nucleus. It will undoubtedly serve as the classical companion paper to his earlier one on the hypoglossus nucleus. Here we see an analysis, step by step, of the electrical response and its meaning in terms of the propagation of activity along the actual histological structures of the neuron elements in the attempt to learn how the impulses are conducted in the soma when invaded by antidromic activity. It is rare to see physiological data correlated with histological study of the system under investigation. Neurophysiologists would do well to emphasize and encourage this mode of research.

The classical paper of Barron and Matthews (1938) which in the words of Skoglund, one of the participants, "has. . . . become the Old Testament of electrophysiology of the spinal cord" was the focus of several papers and much discussion. There is no doubt that this work, by its introduction of the electronic lead and its wealth of basic observations, has inspired much work since 1938, as evidenced by this symposium, and will continue to do so for a long time to come.

To mention another outstanding paper from the list of many, Brenner presented data demonstrating most beautifully the physiological importance of the field in producing synchronization of neurons along the cord during strychnine tetanus. This phenomenon definitely establishes the rôle of the electric field per se as quite distinct from humoral interaction between cells which clearly can be only of short range.

The breadth and wealth of material contributed by leading, established workers as well as by new recruits to neurophysiology, make this volume well worth the study of all persons critically interested in this field.

A. MAURO

PROGRESS IN BIOPHYSICS AND BIOPHYSICAL CHEMISTRY. Vol. 3. J. A. V. Butler and J. T. Randall, Eds. London, Pergamon Press, and New York, Academic Press, 1953. 386 pp. $9.50.

This is the latest volume in an invaluable compendium of review articles written by authorities in the field of biophysics. The nine articles are listed below, with brief comments on the contents of each paper.

"Autoradiography" by I. Doniach, Alma Howard, and S. R. Pelc has introductory sections on the physical principles of autoradiography and on the radiation dose from radioisotopes before the main section, which reviews the published biological and medical applications of the method.

"Polarized Ultraviolet Microspectrography and Molecular Structure" by W. E. Seeds discusses first the theory and the methods, then the results which have been obtained, with particular emphasis on nucleic acids.

"The Infra-red Spectra of Biologically Important Molecules" by R. D. B. Fraser covers the physical connection between infra-red adsorption bands and various bonds before describing recent work, and has a very useful table of references to published material on infra-rad absorption of biological molecules.

"Some Physico-chemical Studies on Viruses" by Roy Markham covers centrifugal and light and electron microscope methods, concluding with a long survey of work on virus crystals.

"Mechanisms of Biological Action of Ultraviolet and Visible Radiations" by Maurice Errere packs over 275 references to the literature into a compactly written paper covering just about very aspect of the problem.

"Recent Work on the Application of the Theory of the Ionic Double Layer to Colloidal Systems" by F. Booth is an exceptionally clear statement of the present state of the theory of colloidal stability, followed by briefer outlines of the theoretical treatment of many other colloidal phenomena, such as thixotropy, electrophoresis, etc.

"Microspectrometry of Living and Fixed Cells" by H. G. Davies and P. M. B. Walker is devoted largely to a careful survey of the physical methods involved.

"Methods of Determining the Form and Dimensions of Particles in Solution: A Critical Survey" by C. Sadron is just that, with emphasis on hydrodynamical (such as viscosity) and light scattering methods.

"Transport Process and Electrical Phenomena in Ionic Membranes" by Torsten Teorell discusses the "passive" transport processes—i.e., with no metabolic steps involved—largely from the standpoint of Teorell's recent theory of electrically charged membranes.

The reviews are comprehensive, with good coverage of the literature down to 1951 and many 1952 references. The articles are clear and readable by the non-expert in the field, and can well be assigned as reading for graduate students. Any serious biophysicist should have this book for his own.

FRANKLIN HUTCHINSON

SUBMICROSCOPIC MORPHOLOGY OF PROTOPLASM. By A. Frey-Wyssling. 2d English Ed. Houston, Elsevier, 1953. 411 pp., 181 figs. $8.00.

This is really the third edition of Frey-Wyssling's famous monograph, for the first English edition (1948) was actually a revision of the original German edition (1938). Following an orienting introduction, the book is divided, as in previous editions, into three parts: fundamentals of submicroscopic morphology, the fine structure of protoplasm, and the fine structure of protoplasmic derivatives. In the first section the author takes up the general principles of structure in colloidal systems with particular emphasis upon methods, information, and theories which are useful in understanding the submicroscopic structure of living systems. The numerous techniques for studying submicroscopic structure are explained in sufficient detail so that the uninitiated may grasp the basic principles of the methods. The second part includes a penetrating analysis of cytoplasmic and nuclear structure. The author is particularly concerned with the morphology of the cell sap, the intracellular group substance in which the formed elements of the cell are suspended. His brief discussion of the formed elements of the cytoplasm is limited to work done largely before 1950. This is unfortunate because, although the author in his foreword states that the third edition is centered on the results of electron microscopy, the most important advances in electron microscopy of cytoplasm have come since 1950 and are perforce excluded from the book. As any book in a rapidly advancing field in out of date by the time of publication, this omission is probably unavoidable; but it is disappointing that Frey-Wyssling could not have included a discussion of the submicroscopic structure of mitochondria and of the ergastoplasm, which was just becoming known in 1951 and 1952 and the characterization of which is still not completed. Here is an enviable opportunity to unite chemical, physical, and morphological principles, as Frey-Wyssling has already so competently done for the cell membrane, the chromosomes, and the erythrocyte. The third part of the book takes up many of the classical objects of submicroscopic morphology, such items as cellulose, chitin, starch grains, silk, keratin, striated muscle fibers, and myelin.

The book is clearly written, and although closely reasoned, is not too difficult to read. The translation (by May Hollander) is considerably better than that of the first English edition. There is an extensive bibliography and an adequate index of authors and subjects. This book is an extremely valuable discussion of the subject which should be readily available to all students of morphology in biological systems, whether chemists, physicists, or microscopists. As Frey-Wyssling writes on the last page of his "retrospect": "The substratum in which life is inherent is not a disperse phase with individual particles or ultramicrons; it possesses a *structure*. Its active centres, which control development, are arranged in a given order. They are not intermingled by mere laws of chance and Brownian molecular movement; the fact is rather that they arrange themselves into a delicate, very plastic and flexible pattern, actuated, as it were, by a purposeful, coordinative impulse. No more than leaves, blown by autumnal winds from the twig and fluttering helplessly in the air, are able to assimilate for the parent tree; can independent, ambulant, reactive molecules take part in any

organized work. It is not surprising, therefore, that the active groups of the enzymes should only be capable of acting in association with a carrier of a given structure. For, orderly biological processes are unthinkable without presupposing structure, and it is therefore out of the question that any living constituent of protoplasm could consist of structureless, fluid, independently displaceable particles. It is for this reason that colloid chemistry, based, as it is, upon the disperse principle, has thrown so little light upon the submicroscopic structure of protoplasm. For the cell certainly is not a pouch filled with ultramicrons suspended in a fluid, whirling about haphazardly and in confusion; it is, on the contrary, a wonderful system, the intrinsic structure of which, could it but be seen, would assuredly fill every observer with an enthusiasm equal to that which microscopic cytomorphology inspires."

SANFORD L. PALAY

THE NATURE OF VIRUS MULTIPLICATION. By Paul Fildes and W. E. Heyningen. New York, Cambridge University Press, 1953. 320 pp. $6.50.

This volume is the record of a symposium of the Society of General Microbiology held at Oxford in April 1952. Each of the sixteen chapters consists of a formal presentation followed by references and the discussion from the floor (with some post-symposium editing).

The nature of virus multiplication is a highly controversial subject with which to deal and yet of fundamental importance in virology. The authors have had to define and interpret such things as virus precursors, intermediate and incomplete forms, virus genetics, the rôle of the host cell, chemistry and physiology, and to struggle with the basic question: are viruses living or non-living?

This far-reaching coverage naturally leads to some speculation and controversy but such was the intent and purpose of the symposium. Here one has the thoughts and ideas of people actively engaged in research and not idle speculation or armchair science. Thus, the reader obtains a summary of the knowledge to date and, more important, an insight into the rationale behind the various experimental approaches to the question of virus multiplication.

WILLIAM H. GAYLORD, JR.

THE MAJOR METABOLIC FUELS. Brookhaven National Laboratory, Upton, New York, 1953. viii + 233 pp. $1.35.

This volume represents the fifth of a series of symposia sponsored by the Biology Department, Brookhaven National Laboratory. The meetings were held in September 1952 and were attended by a number of leading biochemists from this country and from abroad. They contributed to this volume not only by a series of excellent reviews, but also by the stimulating and critical informal discussions which are included in the book.

The reviews deal with the utilization and formation of carbohydrates, fatty acids, and ketone bodies *in vivo* as well as in isolated enzyme systems, with emphasis on recent developments in these areas. The effect of hor-

mones, especially insulin and cortisone, on metabolism is dealt with; attempts are being made to localize the action of these hormones to specific metabolic steps.

The production, disposal, and regulation of blood glucose is reviewed by S. Soskin in an introductory address, while more specific problems of glucose utilization under the influence of hormones are discussed by A. Wick and D. R. Drury, by E. H. Strisower and G. L. Searle, by S. S. Chernick, by R. Levine and M. S. Goldstein, and by D. Stetten, Jr.

Protein as a metabolic fuel is discussed by P. Handler.

Recent advances in the studies of hexose monophosphate oxidations are reviewed by F. Dickens, and a related subject matter, that of gluconate oxidation, is discussed by M. R. Stetten.

The biosynthesis of fatty acids in cell-free systems is reviewed by R. O. Brady and S. Gurin; the metabolism of ketone bodies by W. Sakami and H. Rudney, as well as by S. Weinhouse; the gluconeogenesis from fatty acids by S. Korkes.

The standards of most reviews are very high, and the discussions of some of the controversial issues, such as the influence of hormones on specific enzyme systems, reveal areas of disagreement.

The book, which is available at a nominal price from the Department of Commerce, Washington, D. C., contains several useful reviews of advances in specialized fields of biochemistry. It is regrettable, however, that publication of these papers was delayed for nearly one year, which has made a number of discussions of rapidly advancing fields outdated.

E. RACKER

How To Be Healthy in Hot Climates. By Eleanor T. Calverley. New York, Thomas Y. Crowell Co., 1953. 286 pp. $3.50.

This volume, in its third printing, contains useful information for living in the tropics, including short trips and air travel. It is essentially a handbook for lay persons on the subject of tropical hygience and tropical diseases. There are chapters on various aspects of tropical living all the way from equipment for the traveler to delivering a baby under emergency conditions. It is written plainly for the lay reader, with a minimum of technical language and much common sense, although most of the considerations are treated in some detail.

The author covers such topics as household precautions, food and nutrition, insect control, common ailments and diseases, childbirth, servants, and other topics involved in equatorial existence. There are several charts and maps giving the distribution of diseases, temperature equivalencies, baby formulas, and dietary tables. These general facts about health and disease prevention make this book more than a mere collection of "tips" on how to live comfortably and the information is presented in a manner which is interesting and factual. Personal experience is presented from many years of successful living in the tropics during which the author became an expert on matters of health and disease prevention.

IRA V. HISCOCK

THE INTELLIGENT USE OF THE MICROSCOPE. By C. W. Olliver. 2d Ed. New York, Chemical Publishing Co., 1953. 192 pp., 59 figs., $4.00.

This useful little book was written as a handbook for beginners. In simple, direct language the author presents the principles of image formation and their practical application in the optical microscope. Numerical aperture and resolution are discussed clearly and their importance to the microscopist is emphasized. Instructions for obtaining critical illumination and Kohler illumination are presented along with the reasons behind every step. A useful chapter on the choice of equipment is given, including precautions for the beginner who may be considering the purchase of a second-hand instrument. As this book was written principally for the British public, most of the examples refer to instruments of British manufacturers, but there are sufficient similarities between these and American instruments so that the advice of the author is still applicable. A chapter on filters and another on photomicrography are of most interest to the experienced microscopist. Here the author gives valuable information directly relevant to the problems of the microscopist who attempts to capture in a black-and-white photograph the contrast and detail he sees in his stained preparations. A small chapter on the phase-contrast microscope presents the fundamentals of this newly developed instrument. There is a small reference bibliography of more advanced texts on microscopy and a few citations of the literature of phase-contrast microscopy. An index is provided. This book should be in the libraries of all departments concerned with teaching students.

SANFORD L. PALAY

INDIVIDUAL AND COMMUNITY HEALTH. By William W. Stiles. New York, The Backston Co., Inc., 1953. 466 pp. $6.00.

This volume is intended to acquaint the student with health information which will be valuable to him as a responsible member of the community. It surveys a vast area of knowledge about diseases, sanitation, child care, nutritional disorders, accidents, and related subjects. There is even a chapter on socio-sexual problems. Each of these chapters is broken down into a hierarchy of units which deal with various aspects of the larger headings. Illustrations include statistical charts and tables.

The author's style is reasonably clear; the book is comprehensive, containing a description of many diseases and disorders which are common enough to be of general interest. They are ordered according to causative factors or environments which produce them. The community is naturally interested in knowing what conditions permit the various diseases to flourish in order to guard against them. This idea of preventive as opposed to remedial measures is at the heart of the author's purpose in making this book available. In order to prevent or alleviate unhealthy conditions, it is necessary to be familiar with the medical facts involved. The person who has read this book will have a basic understanding of many problems of the health sciences, and a greater sympathy, perhaps, for those agencies which

have undertaken the task of making our communities healthy places in which to live.

In addition there is a plenum of information which will help the person in attending to his own personal health and that of his family, including a chapter on first aid and another on maternal care. A general knowledge of common disease characteristics will help the student to recognize these conditions and take the proper steps to safeguard himself and others. Even undergraduate college students, by informal exposure, have displayed interest in this volume. The format and the tight, factual manner of presentation have an appeal.

In spite of the lack or small amount of consideration of health education, housing, nursing and occupational health, and inter-relationships in medical care, this volume has so many valuable features that it can be recommended for reference purposes for many college and university groups and for practical health agency executives.

It is hoped that the next edition will give more attention to type and paper so that it will meet the informally developed standards of the National Society for the Prevention of Blindness.

IRA V. HISCOCK

CHROMATOGRAPHIC METHODS OF INORGANIC ANALYSIS WITH SPECIAL REFERENCE TO PAPER CHROMATOGRAPHY. By F. H. Pollard and J. F. W. McOmie. New York, Academic Press, Inc., 1953. viii + 192 pp. $5.50.

This little book begins with a brief and well-balanced history of chromatography. This is followed by a chapter called "Principles of Adsorption and Partition Chromatography" which is so compressed as to be quite superficial—likely to be unsatisfying to the reader who knows the field and of little use to a beginner. The rest of the book is very good, for the authors give quite simply and clearly directions for preparing chromatograms, for locating zones, for analyzing known as well as unknown mixtures of cations, and for separating anions. There is a good chapter on the choice of solvents for separating cations, with an appendix listing some 110 solvent mixtures (which the authors say are all the more useful ones recorded to September, 1952) and R_f values of 24 cations with a number of solvents. Wherever special directions are given there has been care to specify, and give recipes for, the necessary reagents. A chapter on quantitative methods is included, followed by very brief chapters on the use of media other than cellulose as the non-mobile phase, and on electromigration and electrochromatography.

People who write books on chromatography are faced with many difficulties, not the least among which are the colossal literature and the lack of comprehensive theory. The authors have steered a course between these rocks by writing a frankly empirical book, and by limiting themselves almost entirely to paper chromatography and omitting a good deal of literature. They could have omitted a good deal more, including the more gadgeteering aspects of chromatography. However, the book should be very

useful to anyone who is faced with inorganic analyses. The book is well printed and put together—as it should be at 2¾ cents per page. The price seems high even nowadays.

HAROLD G. CASSIDY

CHILD TRAINING AND PERSONALITY. By John W. M. Whiting and Irvin L. Child. New Haven, Yale University Press, 1953. 350 pp. $5.00.

This book is a report of the authors' investigations concerned with the interrelationships between the early experiences of an individual as they affect his personality and the personality characteristics of the individual as they determine the beliefs and practices of his society. They approached this complex question by making use of cross-cultural techniques utilizing the correlational method to test general hypotheses about culture and personality.

The source material utilized in the study consists of extracts from ethnographic reports about the culture of 75 primitive societies, which were selected because the descriptions contained the material the authors felt was necessary to fulfill their criteria. The document representative of our own society chosen for inclusion is the study by Davis and Havighurst (1946, 1947) made in Chicago in the early 1940's.

The methodology employed by the authors and the definition of their terminology is clearly described so that the reader should have little difficulty in placing himself within their frame of reference. This writer feels that inclusion of more descriptive and illustrative material would have made it easier to follow the development of their hypotheses.

For their interpretation of the effect of certain child care practices on personality development, the authors have utilized psychoanalytic theory reformulated in terms of learning theory. Thus they follow a method which promises to be increasingly fruitful in the understanding of personality development: that of restating existing psychoanalytic principles and deriving additional hypotheses from the broad outlines of psychoanalysis, posing them in a form in which they can be tested.

The authors contribute an approach to future research which deserves the careful consideration of those persons interested in this field of study.

The book is not intended to be interpreted as giving advice on child care and training and the reader who is interested in it primarily from this viewpoint is cautioned against trying to take this over into his own child care practices.

SALLY PROVENCE

ESSENTIALS OF PHYSIOLOGICAL CHEMISTRY. By Arthur K. Anderson. New York, John Wiley and Sons Inc.; London, Chapman and Hall Limited, 1953. vi + 480 pp., 42 figures, 11 tables. $5.00.

By virtue of its dynamic nature the science of physiological chemistry is notorious for the vigor with which it resists the setting down in print of its fundamental concepts. Mr. Anderson, in his *Essentials of Physiological*

Chemistry has to an admirable degree overcome this resistance. This, the fourth edition of a tried and proven work which made its first appearance in 1935, adheres to certain structural characteristics which established the success of earlier editions, namely, the avoidance of unnecessary indulgence in controversial topics, since, in the basic stages of study of a subject, "it seems unwise to confuse the student with arguments pro and con"; refusal to submit the reader, as do so many other texts, to the enraging indignity of prolonged discussion of the author's currently cherished topic of research; incorporation and coordination of theory with a description of relevant basic and classic laboratory experiments; a general, not over detailed, yet thorough treatment of the fundamentals of the subject, such that the book is, at the same time, a sufficiently fluent introduction for the beginner in physiological chemistry, as well as a valuable review for the more advanced worker.

Changes in this edition include a rewriting of the chapters on blood, carbohydrate metabolism and enzymes. The sections on chemotherapy, hormones, biological use of isotopes and vitamins have been brought up to date.

In this otherwise well-planned work, it is sometimes disconcerting that the author, while entitling his book *Essentials of Physiological Chemistry*, and defining this subject as "Animal biochemistry, which is an outgrowth of animal physiology," makes numerous and (or so it appeared to this reviewer) unrelated references to the field of phytochemistry.

J. C. C.

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